CHANGES IN THE PROTEINS AND CARBOHYDRATES OF DEVELOPING CEREAL GRAINS AND THEIR RELATIONSHIP TO KERNEL SHRIVELLING IN TRITICALE

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John Stephen Noll

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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

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Grain of three triticale strains, harvested at different stages of maturity, was used to investigate possible relationships between compositional differences and the degree of shrivelling of mature grain. Grain of one cultivar (strain) each of durum wheat, hard red spring wheat and rye was included in the study for comparison purposes. The components that were examined comprised carbohydrates (reducing sugars, non-reducing sugars and starch, including amylose and amylopectin) and proteins (including enzymes).

Reducing sugars content, which decreased with maturation, showed no significant difference between shrivelled- and plump-grained triticales. The non-reducing sugars content of grain showed a similar decreasing trend with maturation as was observed for the reducing sugars. However, the non-reducing sugars content of immature grain was considerably higher in shrivelled triticale grain than in the plump grain. Shrivelled- and plump-grained triticale strains can be readily differentiated on the basis of their starch and amylose contents; shrivelled grain had considerably less starch and amylose. The major factor (not identified) responsible for the lower starch and amylose contents of the shrivelled triticales was established at early stages of kernel development and remained

through to maturity.

The developmental patterns for α-amylase, proteolytic and peroxidase activities reflected differences among the triticales that may be related to shrivelling. α-Amylase activity was higher, throughout the development period, in the two shrivelled strains than in the plump-grained strain. Proteolytic and peroxidase activities, at early stages of kernel development, showed some fluctuation in relation to kernel shrivelling. In mature grain, both enzymes had higher activities in the shrivelled triticales.

Isoenzyme patterns of four enzyme systems (α-amylases, proteases, peroxidases and catalases) were determined for developing and germinated grain. Two groups of α-amylases can be distinguished on the basis of their isoelectric points for both developing and germinated grain. At maturity, both groups of α-amylases had higher activities for the two shrivelled triticale strains than for the plump triticale. Four protease isoenzymes were detected in triticale grain. Each isoenzyme decreased in activity with kernel development. The protease isoenzyme patterns were similar in the three triticales and no distinguishable differences were apparent from the patterns of shrivelled- and plump-grained strains. The peroxidase isoenzyme patterns showed differences in both activity and number of isoenzymes among the three triticales. The shrivelled- and plump-grained strains can be distinguished by the presence or absence of one peroxidase isoenzyme. It is present only in immature and mature grain of the triticale strain that yields plump grain. Two catalase isoenzymes, present in triticale, appear to be similar in the three strains.

Shrivelled and plump triticale grain differed in total nitrogen content; the shrivelled-grained strain had significantly higher nitrogen

content past 12 days after anthesis. The molecular weight distributions of the proteins, as determined by gel filtration and sodium dodecyl sulfate polyacrylamide gel electrophoresis, revealed differences among the three triticale strains. Differences were particularly apparent for each strain among different stages of grain development. However, only minor differences were observed for shrivelled- and plump-grained triticale strains for any specific stage of development. The results of the solubility fractionation experiments revealed only minor differences between shrivelled and plump triticales. However, the electrophoretic patterns of each protein fraction were significantly different and could possibly be used to differentiate shrivelled- and plump-grained triticale strains.

This study showed that shrivelled and plump triticale grain (produced by different strains) differs significantly in some carbohydrate and protein components. Some of these differences are detectable at very early stages of kernel development before kernel shrivelling can be detected visually. Accordingly, these components may possibly be used to select for desirable kernel characteristics in triticale breeding programs.

TABLE OF CONTENTS

TABLE OF CONTENTS - Continued

TABLE OF CONTENTS - Continued

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES - Continued

I. INTRODUCTION

Triticale is a cereal species obtained by combining the genomes of rye (genus *Secale*) and wheat (genus *Triticum*). This man-made interspecies hybrid has presented a potential opportunity for plant breeders to combine the excellent milling and breadmaking qualities of wheat grain with the hardy competitive traits of the rye plant. In achieving this goal, numerous difficulties were faced by the plant breeder. But, its high yielding capability, combined with its superior nutritional quality compared with wheat and its adaptability to environments unsuitable for wheat have been the major factors responsible for the rapid development of triticale from a scientific curiosity into a viable commercial crop. Lorenz (1974) offers an excellent review of the history, development and utilization of triticale from the late nineteenth century to the present.

In order to realize the full potential of triticale as a major cereal crop for human and animal feeding, the factors affecting grain yield and yield stability must be overcome. Partial sterility and kernel shrivelling were the major factors responsible for low yields, which slowed down the development of triticale as a commercial crop (Zillinsky and Borlaug, 1971A). The sterility problem has been essentially overcome but the kernel shrivelling has persisted and remains as the major factor responsible for low grain yields at the present time.

The objective of the present study was to extend the pioneering work of Klassen (1970) on the possible biochemical basis of kernel shrivelling in triticale. The project lends itself to a biochemical

study because of the availability of triticale lines and cultivars which exhibit this abnormal kernel characteristic to different degrees. On the premise that the factors responsible for kernel shrivelling are more likely to be evident during early kernel development, the changes in some carbohydrates, some enzyme systems and the proteins were examined for three lines (which show different degrees of kernel shrivelling) at various stages of maturity. The study, however, emphasizes differences in the grain proteins at the different stages of kernel maturity.

II. LITERATURE REVIEW

The literature review will cover publications on grain shrivelling in triticale and some other grains and the changes in various grain constituents during development (maturation). Because triticale is a relatively new cereal species, there have been only a few reports on the compositional and biochemical changes during grain development. Accordingly, relevant literature on maturing grains will be reviewed. The introductory section will review the historical development of triticale and the literature dealing with the grain shrivelling problem in this species. Subsequent sections will review relevant literature on the carbohydrates, the enzymes and the proteins in maturing (developing) triticale and related cereal grains.

A. Introduction

The fact that rye and wheat can hybridize to form a new species has been known for over a century (Müntzing, 1963). Nineteenth century botanists and plant breeders realized the possibility of combining the vigor and hardiness of rye with the grain quality, productivity and disease resistance of wheat through this hybridization (Briggle, 1969). Depending upon whether a tetraploid wheat (genomes AABB) or a hexaploid wheat (genomes AABBDD) is crossed with rye (genomes RR) either a hexaploid (genomes AABBRR) or an octoploid (genomes AABBDDRR) triticale is produced, respectively. Research effort on triticale was greatly restricted in its early development because of lack of fertility in many of the progenies. In 1934, Müntzing in Sweden initiated the first

intensive research program on triticale (Müntzing, 1939). Working with octoploid triticales, he found that sterility and grain shrivelling were the major obstacles in triticale breeding.

The discovery of the chromosome doubling properties of colchicine (Eigsti, 1938) and the development of effective embryo culture techniques renewed the interest in triticale research on a much broader international scale (Zillinsky and Borlaug, 1971A). In the 1950's extensive research programs were initiated on hexaploid triticales in Canada, Hungary and Spain (Lorenz, 1974). In 1964 a cooperative program on triticale breeding was established between the Department of Plant Science of the University of Manitoba and CIMMYT (International Maize and Wheat Improvement Centre, Mexico). This joint venture has greatly intensified triticale research efforts around the world.

The agronomic and kernel characteristics of triticale have been improved through modern plant breeding and selection techniques (Zillinsky and Borlaug, 1971B). Considerable progress has been made in overcoming the sterility problem and some positive advances have been made in decreasing grain shrivelling. However, to fully realize the grain yielding potential of triticale, it will be necessary to eliminate kernel shrivelling completely.

The superior nutritional quality of triticale over wheat is attributed to its higher protein and lysine contents (Villegas *et al.*, 1970). Because of this factor triticale has received increasing attention in recent years as a staple food for humans. In countries where cereals are consumed in the form of products made from white flour, kernel shrivelling, indirectly has been a major obstacle in the expansion

of triticale's acceptance. Shrivelling greatly affects the milling properties of triticale, resulting in lower yields of darker flour in comparison to wheat. Although the protein content of triticale is generally higher than that of wheat, the protein content of its flour is only equal and often lower than that of wheat flour (Lorenz, 1974). Thus, grain shrivelling is detrimental to the milling quality of triticale grain and thereby limit its commercial utilization in human feeding.

Various approaches have been used in the attempt to find the cause(s) of and ways of eliminating kernel shrivelling in triticale. Plant breeders have attempted to improve kernel characteristics by using only plump seed (separated visually or by various density separators), by treating the seed with mutagenic agents, and by selecting plants for higher fertility (Zillinsky, 1973). Triticale breeders from the earliest time have used visual selection of seed for better kernel characteristics and the best results have been obtained by the application of this technique to the most fertile lines. However, intensive breeding has failed to overcome this abnormality, suggesting that kernel shrivelling in triticale may be the result of incompatible biochemical systems produced by two (rye and wheat) distinctly different genetic components.

In studying several metabolic factors that may influence grain shrivelling, Klassen (1970) concluded that the poor kernel development of triticale is the result of abnormal starch synthesis together with some starch breakdown in the latter stages of kernel development. In this study, it was found that both grain density and α-amylase activity were positively correlated with the degree of shrivelling (assessed

visually). Klassen (1970) also found that, within a single line of triticale, the shrivelled and plump kernels had 32% and 2% aneuploidy respectively, concluding that at least some of the shrivelling might be associated with abnormalities in the chromosome complement.

The first major study of the genetics of seed shrivelling in wheat and triticale was carried out by Darvey (1973). This study showed that three rye and two wheat chromosomes carry genes responsible for major kernel shrivelling. Wheat was classed as a balanced genotype with regards to seed shrivelling because few wheats show any degree of this abnormality until this balance is upset by the removal of specific chromosomes. This genotype balance must occur in rye since both shrivelled- and non-shrivelled-strains exist. Substitution for the chromosomes that carry the shrivelling gene(s) has been suggested as a way to eliminate grain shrivelling in triticale.

The mechanism of seed shrivelling is not known, but the morphological studies by Shealy and Simmonds (1973) suggests that this defect may arise from lesions produced by malformed aleurone and associated peripheral endosperm tissues which are apparent in the early stages of kernel development. Simmonds (1974) postulated that the disruption of normal aleurone layer formation may be due to incorrect programming of degradation of the meristematic layer by cytolytic enzymes. The malformed areas in the aleurone and endosperm tissues can vary from minor distortions to complete absence of sections of tissue. When the grain begins to lose moisture during desiccation in the final stages of maturation these areas collapse and cause shrivelling. Dedio *et al.* (1975) have shown that α-amylase-damaged starch granules are generally

localized in areas where these tissue defects are localized. Frequently in some grains both the aleurone and endosperm cells fail to develop properly in areas at the head of the crease, resulting in endosperm cells that are small, badly distorted and devoid of content (Simmonds, 1974).

The shrivelled-kernel characteristic is not restricted to triticale. It has been also found in other grains such as sorghum (Karper and Quinby, 1963), corn (Jennings and McCombs, 1969), peas (Greenwood and Thomson, 1962) and rye (Darvey, 1973). Differences in some grain constituents have been observed between plump and wrinkled grains, but the cause and effect relationship between the constituent variability and shrivelling has not been established.

B. Carbohydrates

Carbohydrates form the largest fraction of the solid matter of cereal grains. Changes in carbohydrate composition during seed development have been studied in many grains including barley (MacGregor *et al.*, 1971), wheat (Abou-Guendia and D'Appolonia, 1973) and triticale (Klassen *et al.*, 1971). In all cereals studied, the most dramatic changes during grain maturation occurred in the sugar and starch components.

The soluble sugars are generally classed into reducing and non-reducing fractions. In a detailed study of the changes in the carbohydrate components during maturation of wheat, Jennings and Morton (1963) tried to elucidate the relationship between these two sugar fractions. They found that both the reducing and non-reducing sugar contents declined rapidly as a percentage of dry weight increased until midway

through maturity. Afterwards, the decline was less prominent. The marked decline in sugar content at the early stages of grain development coincided with the initiation of rapid starch synthesis. The relative changes in sugar and starch content throughout kernel development was consistent with the precursor-product relationship.

In other cereals, including triticale, Vaisey and Unrau (1964) found that glucose and fructose were the predominant reducing sugars in flour milled from mature grain. All cereals studied so far (barley, LaBerge *et al.*, 1973; maize, Jennings and McCombs, 1969; triticale, Klassen, 1970; wheat, Jennings and Morton, 1963; Abou-Guendia and D'Appolonia, 1972) showed a declining trend in the percentage of reducing sugar content during early growth. Furthermore, Klassen (1970) reported that triticale grain contained more reducing sugars than wheat at equivalent stages of maturity throughout kernel development. The same study showed a slight increase in reducing sugars content in triticale at the late stages of maturation and attributed this increase to high α-amylase activity. He obtained a significant correlation between α-amylase activity and reducing sugar content and between α-amylase activity and the degree of kernel shrivelling. Karper and Quinby (1963) demonstrated that sugary sorghum seeds that wrinkled with maturation contained at least twice as much total sugar throughout kernel growth as did normal seeds. In studying the growth of maize kernels, Jennings and McCombs (1969) found no significant differences in reducing sugar content of kernels from normal and skrunken lines.

The changes in non-reducing sugar content during kernel development showed greater variability among cereals than was observed for

reducing sugars. Abou-Guendia and D'Appolonia (1972) confirmed the earlier results of Jennings and Morton (1963) that in maturing wheat the percentage of non-reducing sugar content showed a gradual decline with kernel maturation. However, on a weight per kernel basis, both reducing and non-reducing sugar contents remained essentially constant during grain development and growth. In maturing barley grain, the percentage (dry basis) of non-reducing sugars was constant (LaBerge *et al.*, 1973). However, this study showed that extracts of grain at early stages of maturation contained large amounts of fructosan material which was not included in the percentage of non-reducing sugars. The amount of fructosan fraction showed a decreasing trend with maturation.

The higher sugar content of triticales relative to other seeds was ascribed chiefly to their higher sucrose content (Vaisey and Unrau, 1964). Sucrose appeared to be the predominant non-reducing sugar in most cereals. In addition, Vaisey and Unrau (1964) found that a large part of the total soluble sugars in wheat, rye and triticale flours were oligosaccharides which yielded only glucose upon hydrolysis.

Jennings and McCombs (1969) studied kernel growth in lines of maize that yielded plump and shrunken kernels. Each kernel type comprised two lines, one with normal and the other with sugary seeds. Non-reducing sugar content reached a maximum level about 15 days after pollination in the plump-seeded lines but then declined significantly by full maturity. In the shrunken-seeded lines, maximum non-reducing sugar level was reached between 18 and 21 days after pollination and a large proportion of this amount remained through to maturity. For each kernel type, there were no differences in sugar contents between

the normal and sugary lines.

Cereal starches are composed of linear (amylose) and branched (amylopectin) glucose polymers. The ratio of these two types of starch molecules appears to be genetically controlled (Zuber, 1965). Bice *et al.* (1945) examined some properties of starch from wheat grain harvested at different stages of development. Considerable amount of starch was found in the grain by eight days after pollination. The increase in starch content during maturation was approximately linear. The amylose to amylopectin ratio increased during maturation indicating that in the early stages of starch deposition amylopectin synthesis is more rapid relative to that of amylose. The pattern of starch accumulation during seed development in other cereals, including triticale (Klassen *et al.*, 1971), was similar to that reported by Bice *et al.* (1945).

Starch content of grain was determined, at various stages of kernel growth for five triticale lines, by Klassen (1970). For mature grain, a significant correlation was obtained between starch content and grain density and between grain density and the degree of grain shrivelling. Starch development profiles indicated a premature cessation of starch accumulation in the lines with severely shrivelled grain. Even in the better lines, starch accumulation appeared to cease at an earlier stage in grain development than in wheat. It was concluded that kernel shrivelling resulted at least partly, from abnormal starch synthesis.

The starch content of kernels from shrunken maize lines was significantly lower than that of kernels from normal maize lines throughout

grain development (Jennings and McCombs, 1969). In a similar study on developing peas, Greenwood and Thomson (1962) found that wrinkled peas had a considerably lower starch content in comparison with a smooth variety. In addition, the wrinkled-pea variety showed an almost two-fold higher amylose content at all stages of growth relative to the amylose content of smooth peas. Thung (1970) concluded that amylose content can be used to differentiate between smooth- and wrinkled-pea varieties.

C. Enzymes

1. α-Amylase

Of the cereal enzymes, the amylases have been most extensively characterized because of their direct involvement in seed maturation and germination. One of the first reports on the variation in activity of an unspecified amylase during the maturation of wheat was by Bach *et al.* (1926). An intensive study of the changes in amylase activity of grain during development and maturation of several cereals (barley, oats, rye and wheat) was carried out by Chrzaszcz and Janicki (1936). Three amylolytic functions were found at all stages of growth in all the cereal species examined.

Sandstedt and Beckord (1946) showed that the total amylolytic action in developing wheat grain was due to α- and β-amylases. They also showed that β-amylase activity increased throughout grain development. The pattern of α-amylase development was in agreement with the earlier work on maturing cereals; the activity increased to a maximum level during early kernel development and then decreased with maturation.

Recent work on developing barley, oats, triticale and wheat grain (Meredith and Jenkins, 1973; Jenkins and Meredith, 1975) confirmed this pattern of α-amylase development which is now accepted to be the pattern for all cereals.

In maturing triticale grain the variation in α-amylase activity was similar to that in other cereals, except that at about 30 days after anthesis a second peak occurred in the activity versus maturation curve (Hill *et al.*, 1973). The second peak was followed by a decreasing trend for all triticale lines that were examined except one, 6A190, which continued to increase in α-amylase activity to full maturity. In an earlier study, Klassen *et al.* (1971) also found this increasing trend in α-amylase activity at the later stages of kernel development in 6A190 and one other triticale line (6A320). Triticales generally had higher levels of α-amylase activity than the wheats and thus resemble more their rye parentage in this respect (Müntzing, 1963). In contrast, Jenkins and Meredith (1975) found that α-amylase activity during kernel development in their triticale was similar to that of developing wheat grain rather than rye grain. On the other hand, the activity of triticale during the post-ripe stage resembled that of rye. Hill *et al.* (1973) showed that shrivelled triticale grain generally had higher α-amylase activity both during development and at maturity than less shrivelled- or plump-grained varieties.

In studying the development of starch granules in maturing wheat, Sandstedt (1946), observed the disappearance of pericarp starch during the early stages of kernel development. This led Sandstedt and Beckord (1946) to investigate the distribution of α-amylase activity in the

maturing wheat kernel. Almost all of the activity was localized in the pericarp layer and only trace amounts were found in the endosperm at all stages of kernel growth. The level of endosperm α-amylase was always low and constant in contrast to the levels of pericarp α-amylase, which increased during early kernel development and then declined with maturation. Sandstedt and Beckord (1946) suggested that the pericarp starch was degraded by the pericarp α-amylase in order to provide nutrients or energy for the growing kernel. Similar results and conclusions were reported for maturing barley (MacGregor *et al.*, 1972) and triticale (Dedio *et al.*, 1975). The activity pattern of the pericarp α-amylase in developing triticale appeared to parallel the pattern for total kernel α-amylase, except that in all the triticales studied including strain 6A190, which yields highly shrivelled kernels, the levels decreased with maturation. On the other hand, endosperm activity was relatively low and constant throughout kernel development in all triticales, except 6A190 which increased dramatically during later stages of maturation. In this respect, the strain 6A190 was abnormal in comparison to other triticale strains and other cereals. The pericarp α-amylase activity was always higher in the shrivelled triticale grain than in the plump grain. Also the optimum activity occurred later in the development of shrivelled lines.

With the onset of germination of cereal grains, the activity of many hydrolytic enzymes, including α-amylase, increased rapidly (Koller *et al.*, 1962). Paleg (1960A; 1960B) showed that gibberellins play a central role in controlling the starch hydrolyzing enzymes of barley endosperm. It is reasonably well established that the increase in

α-amylase activity results from *de novo* synthesis of this enzyme in the aleurone layer. Varner (1964) demonstrated this *de novo* synthesis in germinating barley. These results suggested that α-amylases present in developing and germinating grain of the same strain might be different. In apparent contradiction to this suggestion, Duffus (1969) demonstrated that the same hormone-enzyme relationship exists during grain development and germination. Greenwood and Milne (1968) showed earlier that α-amylases isolated from several cereals had similar molecular weights and catalytic actions, and malting (germination) did not alter these properties.

Lee and Unrau (1969) examined the α-amylases from germinated triticale and its rye and durum wheat parents. The molecular weights were similar, but the kinetic properties of triticale α-amylase were between those of the α-amylases of its parental species. Significant differences were found in the amino acid composition and the electrophoretic mobility of the three α-amylases, suggesting that triticale α-amylase is a new hybrid enzyme, not just a mixture of the amylases from its parents.

Olered and Jönsson (1970) found that germinated wheat contained two types of electrophoretically distinct α-amylases, the "green" or pericarp and the "malt" types. "Malt" type α-amylase appeared only after germination and was not detected in the developing kernels. Both α-amylases comprised a number of molecular forms or isoenzymes.

Isoenzymes are different molecular forms of an enzyme with similar or identical catalytic activities occurring within the same organism. The first report of the existence of α-amylase isoenzymes in germinated

barley was published by Frydenberg and Nielsen (1965). Nine amylase isoenzymes were found, five of which were α-amylases. Subsequently α-amylase isoenzymes of other germinated cereals were studied and characterized (rice, Tanaka *et al.*, 1970; rye, Wagenaar and Lugtenborg, 1973; wheat, Kruger, 1972B).

The existence of α-amylase isoenzymes in developing grains has not been documented as well as the isoenzymes in germinating grain. Chao and Scandalios (1969) observed no variation in the amylase zymograms of maize kernels at various stages of development. Kernel development in barley (Stoddard, 1971) and wheat (Kruger, 1972A) was characterized by gradual changes in the amylase isoenzymes. After reaching maximum activity in the early stages of kernel development, all the α-amylase isoenzymes decreased in activity with maturation. Immature barley α-amylase occurs as a single molecular type (MacGregor *et al.*, 1974). Kruger (1972A) found three isoenzymes in maturing wheat. These three isoenzymes corresponded to the pericarp or "green" α-amylases of Olered and Jönsson (1970).

The three isoenzymes of immature wheat were extensively characterized by Marchylo *et al.* (1976). The isoenzymes were isolated and purified by ion-exchange chromatography. They had similar properties with the exception of their isoelectric points, which ranged from 4.65 to 5.11. Immature wheat α-amylase isoenzymes differed from the germinated-wheat isoenzymes in having broader pH optima, greater heat labilities, lower isoelectric points and higher molecular weights. The isoelectric points of germinated-wheat α-amylase isoenzymes ranged from 6.05 to 6.20 (Tkachuk and Kruger, 1974). Thus, the α-amylase

isoenzymes in developing and germinated wheat have markedly different isoelectric points.

2. Proteolytic Enzymes

The presence of proteolytic enzymes in wheat had been known since the late nineteenth century (Hildebrand, 1946). Most of the research on cereal proteases has been restricted to wheat because of their possible effect on dough properties. Jorgensen (1936) had demonstrated the existence of latent proteolytic enzymes in wheat flour. These proteases could be readily activated by reducing agents such as glutathione and inhibited by oxidizing agents. It had been suspected that potassium bromate, an oxidizing agent, exerted its improving effect in dough by inactivating flour proteases. In contrast, Hites *et al.* (1953) showed that wheat proteases are not affected by either reducing or oxidizing agents and that the low activity in flour from sound wheat is of little or no importance in breadmaking technology.

Results of Hanford (1967) confirmed earlier findings of McDonald and Chen (1964) on the existence of more than one wheat protease. The results of Chua and Bushuk (1969) suggest that there are at least four wheat proteases in germinated wheat. Kaminski and Bushuk (1969) demonstrated, by starch gel electrophoresis, the presence of four proteolytic isoenzymes in wheat flour. However, these latter workers did not rule out the possibility that the different proteases may be aggregates with various other proteins and thereby differ in electrophoretic mobility to give rise to a large number of apparent isoenzymes.

Data on the changes in proteolytic activity during kernel growth and maturation are limited. Bach *et al.* (1926) found that proteolytic

activity in wheat increased during early kernel development to a maximum and then progressively decreased to a very low level at maturity. Bushuk *et al.* (1971) examined the changes in proteolytic activity of three wheat varieties during maturation, which differed widely in baking quality. All three varieties were characterized by a gradual decrease in activity throughout maturation. The high baking quality hard red spring bread wheat had lower protease levels during kernel development and at maturity than the durum and white winter wheats.

Kruger (1973) carried out an intensive study of the changes and localization of the proteolytic enzymes during growth and maturation of two hard red spring wheat varieties. Azocasein and α-benzyl-L-arginine-p-nitroanilide (BAPA) were used as substrates to measure proteinase and peptidase activities, respectively. In both wheat varieties, proteinase activity increased during early kernel growth to a maximum and then decreased to a constant low level at maturity. The greatest part of the activity was localized in the branny layers of the wheat grain. Activity in the endosperm was low and constant throughout the development period. Changes in the peptidase activity during growth and maturation followed a similar pattern as the proteinase activity, except that it was localized largely in the endosperm. Kruger (1973) speculated that the role of the pericarp proteinase is to break down the pericarp protein, prior to the subsequent translocation of these products to the developing endosperm.

Proteolytic activity of mature triticale grain was investigated by Madl and Tsen (1973). Its pH optimum was 4.5; the same as for wheat and rye proteases. In contrast, Singh and Ramsita (1976) found the pH

optimum to be 3.8 for their proteolytic enzyme preparation from triticale. The pH optima of cereal grain proteases, although highly dependent on the assay conditions, are generally in the acid pH region and therefore these enzymes are referred to as acid proteases. In addition, activity was found in all four protein fractions isolated from triticale flour by the Osborne solubility fractionation technique, similar to the findings on wheat protein fractions by Kaminski and Bushuk (1969). Triticale and rye had higher proteolytic activities in both the bran and endosperm fractions compared to wheat. All mill fractions of triticale contained protease activity, but most of it was localized in the non-endosperm fractions. The above review of the proteases from triticale shows that these enzymes are similar to wheat and rye proteases in their properties and distribution in the grain.

3. Peroxidases

Peroxidases are enzymes which catalyze the decomposition of hydrogen peroxide into water and oxygen in the presence of hydrogen donors such as, phenolic substances, cytochrome C, nitrite, leuco-dyes, ascorbic acid, indole compounds, amines and certain inorganic ions (Saunders *et al.*, 1964). The existence of these enzymes has been known for over a century. They are abundantly distributed in a great variety of plants, including cereal grains (Sullivan, 1946). The biological functions of peroxidases are not understood. They have been implicated in roles such as in ion transport, cell wall synthesis, ethylene formation, hormone oxidation (Lamport, 1970) and cell elongation (McCune, 1961). Their possible involvement in dough chemistry through the oxidation and polymerization of flour proteins aroused the interest of

cereal chemists. Honold and Stahmann (1968) examined the activity of this group of enzymes in flour milling fractions of hard red winter (HRW) and hard red spring (HRS) wheats. Most of the activity was found in the bran and germ fractions. The activity of the endosperm was low. HRS wheat fractions were higher in activity than the equivalent HRW wheat fractions.

There have been only a few reports on the changes in peroxidase levels during kernel development and growth. Bach *et al.* (1926), in studying a number of enzymes in developing wheat grain, also included peroxidase. They observed that peroxidase activity reached a maximum level in early kernel growth and then remained constant throughout maturation. More recently, Kruger and LaBerge (1974) examined the changes in peroxidase activity during maturation of one variety of durum wheat and one HRS wheat. In both wheats, peroxidase activity increased to a maximum level at about 30 days after flowering and then gradually decreased to the relatively low activity of mature grain. In early development, the activity was localized mainly in the pericarp and cross-celled layers. As the grain matured, activity was detected in the aleurone, endosperm, scutellum and embryo and increased with maturation. Results of Honold and Stahmann (1968) and Kruger and LaBerge (1974) showed that at maturity most of the peroxidase activity of wheat was localized in the bran and germ fractions.

Peroxidases, like most other enzymes, exist in a number of different molecular forms or isoenzymes. One of the earliest reports that cereal peroxidases exist as isoenzymes was that of Tagawa and Shin (1959). They showed that purified peroxidase, isolated from wheat germ, comprised

of two electrophoretically distinct components. Because they are widely distributed and easily extracted and detected, peroxidase isoenzymes have been investigated more extensively than any other plant isoenzyme system (Scandalios, 1974). The number of isoenzymes detected for a particular enzyme extract seems to depend on the conditions of separation and on the nature of the hydrogen donor used for detecting peroxidase activity. LaBerge *et al.* (1973) used five different hydrogen donors to detect peroxidase isoenzymes of mature barley kernels. Both the number of isoenzymes and the zymogram patterns obtained depended on the particular hydrogen donor used. The number of isoenzymes detected was eight to 12 in maize (Scandalios, 1969), 14 in barley (LaBerge *et al.*, 1973) and 12 in wheat (Kruger and LaBerge, 1974).

Kruger and LaBerge (1974) found that the number and the relative concentration of peroxidase isoenzymes varied among different tissues and with the stage of kernel development. Ockers *et al.* (1966) and Harmey and Murray (1968) suggested that the concentration of the various peroxidase isoenzymes may be under hormonal control. According to Brewer (1970) the broad substrate specificity of this group of enzymes indicates an extremely diverse functionality which could include hormone action, tissue differentiation and development.

D. Proteins

1. Introduction

Proteins are the second largest constituent, after carbohydrates, of cereal grains. Osborne (1907) classified cereal proteins into four fractions depending on their solutility; water-soluble albumins, salt

solution-soluble globulins, alcohol-soluble prolamins, and acid- or alkali-soluble glutelins. Some of these fractions have been given crop specific names. For example, in wheat prolamine is called gliadin and its glutelin is glutenin. The prolamines of barley and maize are called hordein and zein, respectively.

The proteins of wheat endosperm have been studied extensively because of their involvement in the breadmaking quality of wheat flour. Dronzek *et al.* (1970) suggested that the breadmaking quality of bread flour depends on a critical balance of the different proteins present in flour. Orth and Bushuk (1972) fractionated the flour proteins of twenty-six wheat varieties of diverse baking qualities and found a quantitative relationship between breadmaking quality and the protein solubility distribution. They showed that the amount of glutenin was inversely related to loaf volume while the amount of insoluble residue protein and loaf volume were directly related.

Numerous other solvent systems, including various concentrations of urea (Lee and MacRitchie, 1971) had been proposed to extract and fractionate cereal proteins, especially the gluten proteins. In spite of some of its shortcomings, no other fractionation procedure has been used as widely as the classical Osborne procedure or its numerous modifications. The procedure, as modified by Chen and Bushuk (1970A), has been extensively used in our laboratories to fractionate, wheat, rye and triticale proteins (Dronzek *et al.*, 1970; Orth and Bushuk, 1972; Tanaka and Bushuk, 1972).

Cereal proteins can be fractionated by a variety of methods other than those based on solubility. Woychik *et al.* (1960) fractionated

wheat gluten proteins by ion exchange chromatography using 0.01N acetic acid as the initial solvent and a pH gradient from 3.4 to 1.5 for the elution. Jones *et al.* (1963) obtained six fractions from wheat gluten dispersed in a variety of solvents and separated by gel filtration on Sephadex G-75. Meredith and Wren (1966) used a strongly dissociating solvent comprising of acetic acid, urea and cetyltrimethylammonium bromide to obtain a molecular-weight distribution profile of wheat flour proteins. Four major peaks, representing glutenin, gliadin, albumin and non-protein components were obtained. Sodium dodecyl sulphate polyacrylamide-gel electrophoresis (SDS-PAGE) was introduced by Shapiro *et al.* (1967) as a simple and rapid technique of estimating the molecular weights of proteins in a complex mixture. SDS-PAGE has been widely used to study gluten proteins of wheat, rye and triticale (Bietz and Wall, 1972; Orth and Bushuk, 1973; Orth *et al.*, 1974; Preston and Woodbury, 1976).

The heterogeneous nature of cereal proteins can best be demonstrated by various forms of electrophoresis. Elton and Ewart (1962) found profound differences in the electrophoretic patterns on starch gel of protein extracts of different cereals. Various protein fractions (of cereals including triticale) obtained by solvent extraction have been further characterized by electrophoresis (Kaminski, 1962; Chen and Bushuk, 1970C), gel filtration (Chen and Bushuk, 1970B), electrofocusing (Wrigley, 1968) and immunological techniques (Ewart, 1966).

2. Non-Protein Nitrogen

Woodman and Engledow (1924) and McCalla (1938) observed a gradual decrease in the percentage of non-protein nitrogen during development

of wheat grain. Similarly, Jennings and Morton (1963) found that, in maturing wheat, the non-protein nitrogen formed a high proportion of the total nitrogen during early kernel growth. The proportion declined rapidly from day 8 to day 19 after anthesis and then more slowly during the later stages of maturation. The amount of non-protein nitrogen, per grain, remained almost constant during development. Similar trends in non-protein nitrogen with grain development were observed for rice (Palmiano, 1968), barley (Ivanko, 1971) and triticale (Dexter and Dronzek, 1975A).

The non-protein nitrogen content of maturing triticale was intermediate of its rye and durum wheat parents (Dexter and Dronzek, 1975A). However, triticale resembled its rye parent by having a higher proportion of this nitrogen fraction at the early stages of kernel growth. Free amino acids of developing grain formed a major portion of the non-protein nitrogen content for the three cereals studied by Dexter and Dronzek (1975C). The pattern of changes in non-protein and protein nitrogen resembled the precursor-product relationship that exists between reducing sugars and starch in developing grain.

3. Soluble Proteins (Albumins and Globulins)

Cereal proteins can be divided into two broad groups, the first is the group of proteins that are soluble in dilute salt solutions and the second comprises the insoluble group. The soluble proteins are composed mainly of the water-soluble albumin and the salt-soluble globulin proteins, together with some glycoproteins, nucleoproteins and other lipid-protein complexes (Kasarda *et al.*, 1971). This albumin-globulin protein fraction contains most of the enzymes. The insoluble

group comprises the storage or gluten (gliadin and glutenin) proteins.

The early work of Woodman and Engledow (1924) and McCalla (1938) showed that the amount of the soluble protein per grain of wheat remained constant during the development period. In contrast, Graham *et al.* (1963) and Jennings (1968) found that the amount of soluble protein increased with kernel growth. However, on the percentage of solids basis this fraction decreased with maturation to the constant low level of mature wheat. In rice, the amount of the soluble fraction showed some fluctuation during kernel development (Palmiano *et al.*, 1968). It increased during the first two weeks of ripening, reached an optimum level between the second and third weeks after anthesis and then decreased progressively toward maturity. Ivanko (1971) found that in developing barley kernels the amount of the soluble fraction showed a slight increase when expressed on a per grain basis. The percentage of albumin showed little variation throughout grain ripening, whereas the percentage of globulin decreased.

Dexter and Dronzek (1975A) studied the change in the protein solubility distribution in triticale and its rye and durum wheat parents during kernel development. Two weeks after flowering, the durum wheat contained the largest proportion of albumins of the three cereals, but then decreased to such an extent that at maturity its albumin content was below that of rye and triticale. The proportion of albumin in rye increased with kernel growth. The proportion of albumin in triticale was essentially intermediate between the values for the parents throughout the kernel development period. The proportion of globulin protein, which varied slightly during grain development, was approximately the same for the three cereals.

The rate of synthesis of the Osborne protein fractions during kernel growth in triticale and its parental species was measured by Dexter and Dronzek (1975A) in terms of the rate of incorporation of ^{14}C-labelled leucine into each protein fraction. All fractions incorporated labelled leucine throughout kernel development. The amounts of labelled leucine incorporated into the albumin were quite different for the three cereals. It was concluded that the triticale and the durum wheat synthesized albumin protein most rapidly between the first and second week after flowering; then the rate of synthesis progressively declined and levelled-off at maturity. In rye, the synthesis of the albumin fraction increased rapidly between the second and third weeks after flowering and remained at a relatively (to durum wheat and triticale) constant level to maturity. The amount of labelled leucine incorporated into the globulin remained essentially constant for the three cereals investigated.

Graham and Morton (1963) examined the changes during kernel development in the soluble proteins of wheat by starch gel electrphoresis. Their results indicated that the amounts of the protein components in the albumin-globulin fraction were similar at different stages of growth. Subsequent results of Graham *et al.* (1963) and Feillet (1965), showed that the changes in the soluble proteins during grain development of wheat were quantitative rather than qualitative. This agreed with the earlier results of Graham and Morton (1963).

Using the more sensitive immunoelectrophoresis technique, Rainey and Abbott (1971) demonstrated the development of seven new salt-soluble protein components in wheat during the late stages of kernel maturation.

Their soluble fraction appeared to change in antigenic properties during grain ripening.

The earlier findings of Hall (1959) was supported by the results of Yong and Unrau (1964), that tirticale contained some new or hybrid protein components not found in either parent. Starch-gel electrophoresis revealed both quantitative and qualitative differences in equivalent protein fractions of mature triticale grain and its parental species (Yong and Unrau, 1964). Many protein components from the three related species showed the same electrophoretic mobility. Later, the more detailed investigations by Chen and Bushuk (1970C) and Dexter (1974), did not detect (by polyacrylamide disc-gel electrophoresis) any new proteins in triticale that were not present in the rye or the durum wheat parent. Both studies noted qualitative and quantitative species differences in the corresponding albumin and globulin fractions.

Dexter (1974) used polyacrylamide electrophoresis to demonstrate both quantitative and qualitative changes in the albumin and globulin components during kernel maturation in triticale.

Chen and Bushuk (1970B) used gel filtration to determine the molecular weight (MW) distribution of the Osborne solubility fractions of the proteins of triticale, its durum wheat and rye parents and one variety of HRS wheat. The elution profile for the albumins had four distinct peaks (fractions), ranging in MW from 6,000 to over 150,000. The two high MW fractions appeared, by electrophoresis, to be gliadin and glutenin contaminants. The two fractions in the range from 6,000 to 23,000 were considered to be the albumins. The globulins separated into three major groups corresponding to MW's of 8,000 to 20,000 and greater than 150,000.

There have been no reports on the MW distributions of the soluble proteins of cereals at various stages of kernel development.

4. Gluten Proteins

The gluten proteins of cereals are composed of two fractions, the prolamins and the glutelins. The term gluten was originally introduced to include the insoluble proteins of wheat only, however it is now used to refer to this group of proteins from all cereal grains. Gluten proteins of wheat have been studied extensively because of their important functional role in the physical properties of bread dough. In terms of the biological role in the seed, gluten proteins are referred to as storage proteins.

Woodman and Engledow (1924) published the first recorded study of the changes in gluten proteins during the maturation of wheat. Small amounts of both gliadin and glutenin were found at very early stages in kernel development. The amounts of both fractions increased with maturation. However, the gliadin fraction appeared to be synthesized at a faster rate than the glutenin during grain ripening. A similar pattern of change in the gluten components was observed by McCalla (1938), Graham *et al.* (1963) and Feillet (1965) in maturing wheat grains. At maturity, the gluten comprised approximately equal amounts of gliadin and glutenin components (Pomeranz, 1971).

Bushuk and Wrigley (1971) observed, by gel filtration, both quantitative and qualitative differences in the glutenin fraction during development of wheat grain. Glutenin was present at all stages of kernel development after 14 days after flowering. The glutenin of bread wheats had a characteristic low molecular weight component which appeared during

the late stages of maturity. It was suggested that the low MW glutenin might be important to breadmaking quality since it was absent in the durum and soft wheats. Hoseney *et al.* (1966) had suggested earlier that the optimization of breadmaking properties of wheat with maturation was due to the increase in MW and complexity of the gluten proteins.

In rice, the amount of prolamin and glutelin increased with maturation (Palmiano *et al.*, 1968). In this cereal, most of the storage protein is of the glutelin type.

Ivanko (1971) found that, in maturing barley, the hordein (prolamin) fraction was synthesized at a faster rate than the glutelin, as is the case of wheat. The percentage of hordein increased with maturation while the amount of glutelin decreased.

Dexter and Dronzek (1975A; 1975B) examined the gliadin and glutenin fractions of triticale and its rye and durum wheat parents at various stages of grain maturity using solubility fractionation, electrophoresis and amino acid composition analysis. The amount of each fraction increased with development. During the later stages of development, the proportion of these two protein fractions remained approximately constant for each species; quantitative differences were observed among the three species. All three contained a relatively large, and constant, proportion of insoluble residue protein throughout maturation. The solubility distribution of the gluten proteins for triticale was essentially intermediate to those for the parental species throughout kernel development. These results were in general agreement with the earlier findings of Chen and Bushuk (1970A).

The amount of acetic acid soluble-protein in wheat, gliadins and

soluble glutenins, increased with maturation while the alkali-soluble fraction showed an almost corresponding decrease (Jennings, 1968). These alkali-soluble proteins appeared to be similar or identical, by starch-gel electrophoresis, to the albumin and globulin proteins. Jennings (1968) suggested that a fraction of the storage protein is composed of albumin-globulin-like proteins that had aggregated into insoluble complexes by interacting with other proteins or other substances (e.g. carbohydrates or lipids).

Jones *et al.* (1959) demonstrated the heterogeneous nature of wheat gluten proteins by moving boundary electrophoresis. Four major and one minor gluten components were found, including some albumin-globulin contaminants. Woychik *et al.* (1961) showed, by starch-gel electrophoresis, that wheat gliadin consisted of at least eight components, while the glutenin remained at the origin. Kaminski (1962) detected 18 components in his glutenin preparation but all the components could be accounted for in the albumin, globulin and gliadin fractions. Graham (1963) demonstrated that the soluble proteins of wheat are quite different from the gluten proteins in electrophoretic mobility, but there was some overlapping of the two fractions. Distinct similarities and differences in electrophoretic mobilities were observed between the gliadin and acetic acid-soluble fractions.

Woychik *et al.* (1964) demonstrated that reduction of the disulfide bonds did not increase the number of gliadin components detected by electrophoresis, although the mobilities of the components were reduced somewhat. Reduction of glutenin disulfides released 20 or more components. Some of these glutenin components were identical, by electrophoresis, to the gliadin components. These workers concluded that the

main difference between the gliadins and the glutenins is in the nature of their disulfide bonding. It was postulated that the gliadin disulfides were of the intrapolypeptide type whereas the disulfides of glutenin were of the interpolypeptide type. Elton and Ewart (1966) arrived at a similar conclusion regarding this structural difference between the gliadin and glutenin proteins.

The acetic acid-insoluble gluten proteins of wheat were examined by Cluskey and Dimler (1967) using moving-boundary electrophoresis. This gluten fraction appeared to be similar to glutenin; it had a high molecular weight and did not penetrate the starch gel during electrophoresis. However, in amino acid composition it resembled the albumin-globulin proteins more closely than whole wheat gluten. Similar results were obtained by Jennings (1968).

Graham and Morton (1963) examined various protein fractions of wheat for qualitative differences during maturation. The number and relative amounts of the acetic acid-soluble components (composed of gliadins and soluble glutenins), by starch gel electrophoresis, were similar for different stages of kernel development. In contrast, Feillet (1965) found that the gliadin components showed a characteristic pattern, as detected by electrophoresis, only after 35 days after anthesis. The gliadin fractions of immature samples showed only a few distinct bands; the other bands were streaky and unresolved. This study also showed that varieties of wheat can be identified by the electrophoretic patterns of their gliadins. These patterns can be made still more variety specific by two dimensional separation techniques such as the combined gel electrofocusing-electrophoresis method of

Wrigley (1970) which showed more than forty different components in the gliadin fraction of bread wheat.

Dexter (1974) examined, by polyacrylamide disc-gel electrophoresis, the changes in the gliadin components in maturing triticale and its parental species. Most of the components in durum wheat gliadin were recognizable in extracts of grain harvested two weeks after flowering. Extracts of rye or triticale harvested at the same time did not show any distinct components. All gliadin components were detectable in extracts of grain harvested between the third and fourth week after flowering for the three cereals. Beyond that point in maturity, gliadin patterns changed only in band intensity.

Studies of glutenin proteins have been hindered by the insolubility of this protein. Glutenin subunits can be examined by electrophoresis after reduction of the disulfide bonds (Elton and Ewart, 1966). Furthermore, by using sodium dodecyl sulfate modification of the polyacrylamide-gel electrophoresis technique (SDS-PAGE), it has been possible to determine the molecular weights of glutenin subunits (Bietz and Wall, 1972; Orth and Bushuk, 1973).

In the present investigation the MW distribution of proteins, extracted from grains of various stages of development, will be examined by gel filtration and SDS-PAGE. Reports on the changes in MW's of proteins during grain development are few. Accordingly, a brief review of the pertinent literature on the MW distribution of gluten proteins of mature grain (mainly wheat) is presented in the following paragraphs.

Chen and Bushuk (1970B) examined the MW distribution of gliadin and glutenin proteins (by gel filtration on Bio-Gel P-150), prepared by

the modified Osborne solubility fractionation procedure, of flours of triticale, its rye and durum wheat parents and one variety of HRS wheat. The gliadin fraction ranged in MW from 80,000 to 90,000. The glutenin fraction could not be resolved, presumably because of its high MW, and was eluted in the void volume. Wheat gliadins are single polypeptide chain molecules ranging in MW from 25,000 to 50,000 (Kasarda *et al.*, 1971). This range of MW's for gliadins is more widely accepted than the somewhat higher estimates reported by Chen and Bushuk (1970B).

Rye (Preston and Woodbury, 1975) and wheat (Preston and Woodbury, 1976) gliadin proteins were fractionated into four fractions, by gel filtration on Sephadex G-100 column, with approximate MW's of 10,000, 27,000, 44,000 and greater than 100,000. Significant varietal variation, in the MW distribution of the four fractions, was found for wheat. Non-reduced gliadin components of rye and wheat, as determined by SDS-PAGE range in MW from 10,000 to 300,000. The high MW components (with apparent MW between 150,000 and 300,000), upon reduction of disulfide bonds, yielded a single gliadin component with MW of 110,000 in rye, whereas for wheat three subunits of MW 40,000, 50,000 and 53,000 were obtained. The three lower MW fractions of both rye and wheat, as determined by SDS-PAGE, were composed of single-chain proteins, i.e. their MW's did not decrease substantially upon reduction.

Glutenin proteins, extracted from wheat grain, range in MW from 40,000 to millions (Kasarda *et al.*, 1971). However, the SDS-PAGE patterns of reduced glutenin indicate that glutenin subunits vary in MW from approximately 10,000 to 130,000 (Bietz and Wall, 1972; Orth and Bushuk, 1973). The largest fraction of these glutenin subunits fall

in the MW range 40,000 to 100,000. In native (unreduced) glutenin, the subunits are either linked through intermolecular disulfide bonds to give rise to glutenin molecules with very high MW or aggregate into micelles with high particle weights (Kobrehel and Bushuk, 1977).

Khan and Bushuk (1976) used the SDS-PAGE technique to study glutenin subunit composition of bread and durum wheats at different stages of grain maturity. The type of subunits (in terms of molecular weight) in the glutenin was essentially constant from the earliest stages of kernel development examined (19 days after anthesis) to maturity. It was noted that the relative proportion of some of the subunits changes slightly during maturation.

Glutenin subunit composition of mature triticale grain and its parental species have been determined by SDS-PAGE (Orth *et al.*, 1974). The subunit patterns revealed major differences among the three cereals; each triticale subunit was also present in one or both parental species.

Dexter (1974) investigated, by SDS-PAGE, possible changes in glutenin subunit patterns of maturing triticale and its parents. Both qualitative and quantitative differences between the three species were observed. The stage in development at which the pattern that is characteristic of mature grain was evident differed among the cereals. The "mature" patterns were evident at two, three and four weeks after flowering for durum wheat, triticale and rye, respectively.

The only biochemical study of triticale related to grain shrivelling has been that of Klassen (1970). This investigation concentrated on the carbohydrate components and related metabolic factors. There are no published reports on the proteins of triticale (or other species) in

relation to grain shrivelling. Accordingly, the present investigation was undertaken to examine possible differences in some of the enzymes and proteins of developing grains of triticale strains that produce grain that differs in the degree of kernel shrivelling at maturity. In addition, Klassen's work on the carbohydrate components was re-examined and extended to include additional components.

III. MATERIALS

Three triticale strains that exhibit varying degrees of kernel shrivelling at maturity were selected for the present investigation. Mature grain of the three strains (Fig. 1) show the range of kernel shrivelling. In addition to the three triticale strains, experimental material included the parental species of one of the triticale strains (6A190), Stewart 63 durum wheat and Prolific spring rye and one strain (cultivar) of Canadian hard red spring (HRS) wheat, cv. Manitou. The HRS wheat was included in the study for comparison purposes. The strains that were used and their pedigrees are listed in Table 1.

The six strains (cultivars) were grown together on experimental field plots at the University of Manitoba in 1972. Spikes of all strains were tagged at the time when approximately half of the anthers were extruded; the date when this occurred was taken as the date of anthesis (flowering). Subsequently, a sufficient number of heads were removed at 12, 14, 18, 22 and 26 days after anthesis and at normal maturity (approximately 60 days after anthesis). After removal the heads were immediately frozen. Subsequently, the grain was threshed by hand, freeze-dried and ground on a Wiley laboratory grinder to pass through 60-mesh screen. The ground whole meal samples were stored at -20^{o}C and used for all analysis, except where indicated otherwise.

Flour samples (used in some experiments) were prepared by milling mature grain of all cereals investigated on a Brabender Quadramat Mill. This mill gives a fairly good separation of the endosperm from the bran and germ.

FIGURE 1. Variations in kernel characteristics of mature cereals.

A. Triticale 6A190 - High Shrivelling

B. Triticale 6517 - Intermediate Shrivelling

C. Triticale 6A250 - Essentially no Shrivelling

D. HRS wheat (cv. Manitou)

E. Durum wheat (cv. Stewart 63)

F. Spring rye (cv. Prolific)

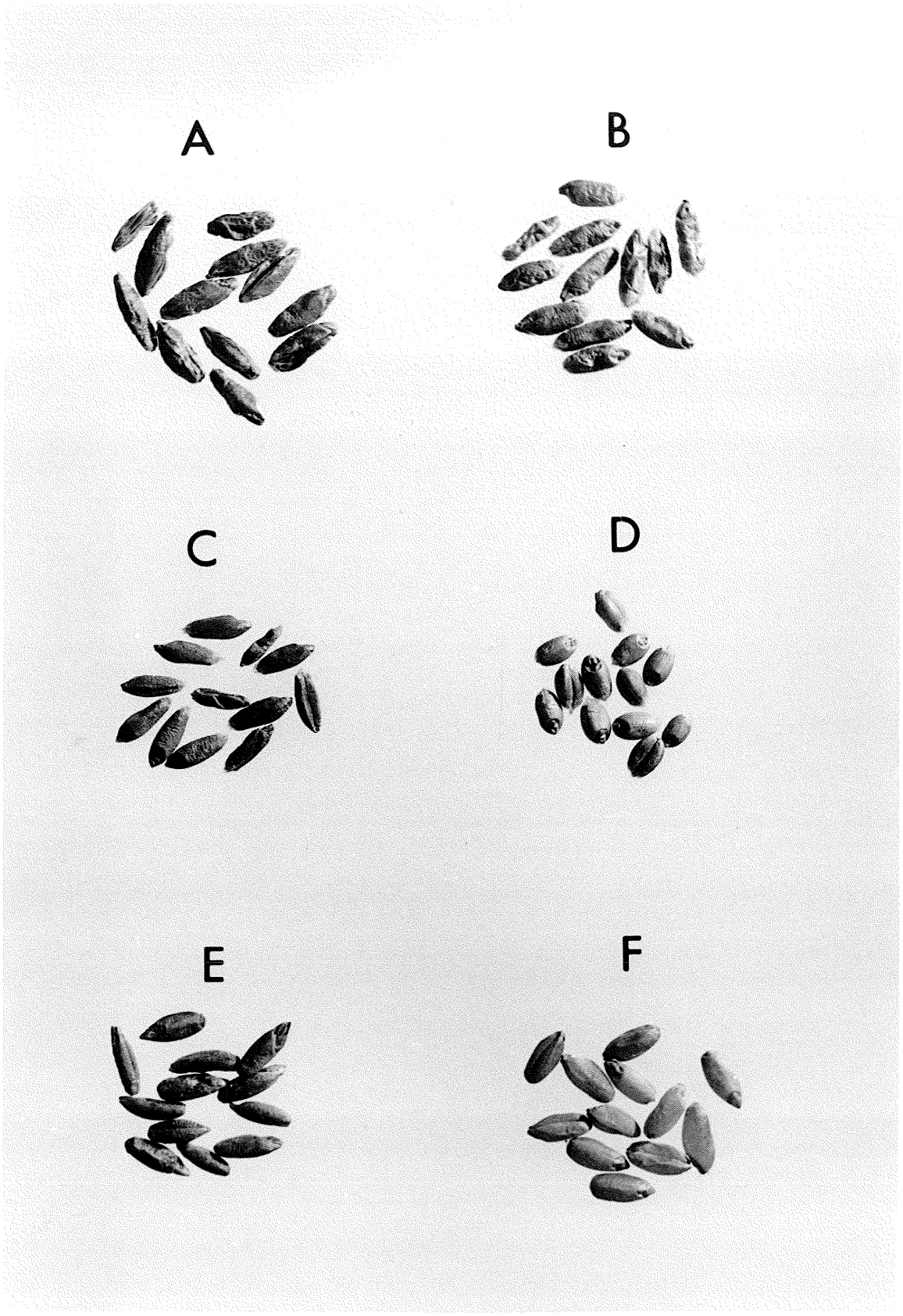
A
B
C
D
E
F

TABLE 1. STRAINS (CULTIVARS) OF CEREAL GRAINS IN THE SHRIVELLING STUDY.

Cereal and Strain	Pedigree
Triticale	
6A250	*T. persicum* x *S. cereale*
6517	*T. durum* (Leucurum) - *S. cereale* (Dw. Petkus) x *T. Persicum* - *S. cereale*
6A190	*T. durum* (Stewart 63) x *S. cereale* (Prolific)
Durum wheat	
Stewart 63	Stewart[8] x St. 464
Spring rye	
Prolific	Selection from an unknown German cultivar.
Hard red spring wheat	
Manitou	(Thatcher[7] - Frontana x Thatcher[6] - Kenya Farmer) x Thatcher[6] - P.I. 170925

Studies were also made on germinated grain. To prevent mould growth during germination, the grain was soaked in a 2% bleach solution for 5 minutes prior to germination. The samples were then extensively washed with sterilized distilled water, soaked in the same water for approximately 12 hours, and germinated in a germination cabinet for 48 hours at 18°C. The germinated grain was frozen and freeze-dried. The rootlets were removed and the grain was ground on the Wiley grinder. The meal was stored at -20°C until used for analysis.

IV. METHODS

A. Physical Characteristics, Moisture and Nitrogen Contents

1. Kernel Volume and Weight

Single kernel volume of mature grain was determined from the volume displacement of a light paraffin oil by 200 kernels.

Single kernel weight at the various stages of development was determined from the weight of 250 freeze-dried kernels.

2. Moisture Content

The kernels were removed by hand from spikes immediately after harvesting and the moisture content was determined on two to five gram samples by the AACC one-stage oven method (1962).

3. Nitrogen Content

Total nitrogen content was determined by micro-Kjeldahl procedure using titanium dioxide as the catalyst (Williams, 1973). The distillate was titrated to an end point of pH 4.5 rather than to a color change end point for improved accuracy (Dexter, 1974).

B. Carbohydrates

1. Sugar Content

a. Extraction. The sugars were extracted by the method of Klassen *et al.* (1971). Ten ml of cold 80% ethanol solution was added to a 100 mg sample in a 15 ml glass centrifuge tube, tightly capped, and mixed on a Multi-Purpose rotator for 15 min in the cold room (4 - 8^{o}C). The mixture was then centrifuged for 10 min at 20,000xg. The residue was extracted similarly two more times. The three

supernatants were combined in a 50-ml volumetric flask and evaporated to a small volume (5 - 6 ml) in a boiling water bath. The extract was cooled and diluted to volume with distilled water. The diluted extract was filtered through glass wool and the filtrate used directly for the determination of reducing sugars content and for the determination of total sugars after hydrolysis.

b. Reducing sugars. Reducing sugars content was determined in duplicate on one ml aliquots of the filtrate (see above) by the neocuproine method of Brown (1961) as modified by Dygert *et al.* (1965) using D-glucose as the standard.

c. Non-reducing sugars. Non-reducing sugars content was determined as the difference between the total sugar content and the reducing sugars content (as assayed above). For total sugar content determination, a 5.0 ml portion of the extraction filtrate was hydrolyzed with 0.5 ml of 3M H_2SO_4 in a boiling water bath for 15 min. The hydrolysate was cooled, neutralized with sodium hydroxide solution, diluted to 25 ml with distilled water and assayed for reducing sugars by the neocuproine method.

2. Starch Content

a. Extraction. The extraction procedure for total starch determination was based on the perchloric acid method of Pucher *et al.* (1948) and was as follows. The residue, remaining after extraction of soluble sugars (above) from 100 mg ground meal, was suspended in 4.0 ml of distilled water in a 15 ml glass centrifuge tube and heated in a boiling water bath for 20 min to gelatinize the starch. The

sample was cooled and 3.0 ml of 70-72% perchloric acid solution was added, the centrifuge tube was tightly capped, and its contents mixed for 20 min on a Multi-Purpose rotator at room temperature (23 - 25°C). The mixture was then centrifuged for 10 min at 1000xg. The supernatant was transferred into a 100 ml volumetric flask and the residue was similarly extracted once more. The second extract, without centrifugation, was combined with the initial extract, neutralized with sodium hydroxide solution, cooled, diluted to volume with distilled water and filtered through glass wool. The filtrate was used for amylose and total starch determinations.

b. Amylose. Amylose content was determined on a 3.0 ml aliquot of the filtrate (above) by the rapid colorimetric method of Williams *et al.* (1970). A commercial high amylose starch containing 50.0% amylose by the amperometric method as described by Williams *et al.* (1970) was used to prepare a standard curve for this colorimetric procedure.

c. Assay for D-glucose. The procedure for the determination of D-glucose was based on a glucose oxidase method (MacGregor *et al.*, 1971); the details of the procedure obtained from MacGregor (personal communication). The assay was carried out in duplicate. Ten ml of the glucose oxidase [Biochemica test combination for blood-sugar (GOD-Perid Method) Boehringer, Mannheim, W. Germany] solution was added to 1.0 ml of starch hydrozylate in a test tube, tightly capped, vigorously mixed on a vortex shaker, and incubated at 35°C for 30 min. The resulting solution was cooled to room temperature (23 - 25°C) and its absorbance measured at 600 nm on a Zeiss PMQ II Spectrophotometer using water as the

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blank. The amount of D-glucose in the aliquot was determined from a standard curve prepared using pure D-glucose. The value was then converted to glucose content per 100 mg of dry grain sample.

d. Starch. Five ml of the extraction filtrate (above) was pipetted into a 50 ml volmetric flask and hydrolyzed with 5.0 ml of 3M H_2SO_4 in a boiling water bath for two hours. The hydrolysate was cooled, neutralized and diluted to volume. D-glucose content of the neutralized hydrolysate was determined by the glucose-oxidase procedure (as described in preceding section). The amount of starch was equal to the amount of D-glucose multiplied by the factor of 0.9 (Hassid and Neufeld, 1964).

C. Enzymes

1. α-Amylase Activity

a. Extraction. A 0.2-g sample was extracted with 30 ml of 0.2M acetate buffer, pH 5.5 containing 10^{-3}M calcium chloride (MacGregor *et al.*, 1971) at 4°C on a Multi-Purpose rotator. After a four-hour extraction period, the extract was filtered through Whatman No. 1 filter paper and the filtrate was used directly in the enzyme activity assay.

b. Assay. The procedure of Briggs (1961) as modified by MacGregor *et al.* (1971) was used for the measurement of α-amylase activity. One ml aliquot of enzyme filtrate was incubated with 1.0 ml of substrate solution (0.05% β-limit dextrin solution of 0.2M acetate buffer, pH 5.5 containing 10^{-3}M $CaCl_2$) for exactly 10 min at 35°C. The reaction was terminated by the addition of 5.0 ml of iodine solution (0.05M HCl

solution containing 0.05% potassium iodide and 0.005% iodine). The assay solutions and appropriate standard and blank solutions were allowed to stand at room temperature (23 - 25°C) for 30 min before their absorbances were measured at 540 nm on a Cary 15 Spectrophotometer. The results, average of duplicate activity measurements, were expressed in IDC units. One IDC unit is the enzyme activity required to lower the absorbance of a standard digest from 0.6 to 0.4 in 100 min.

2. α-Amylase Isoenzymes

a. Extraction. The α-amylases were extracted by homogenizing a 0.25 g sample with 1.5 ml of 0.01M $CaCl_2$ solution (Kruger, 1972A) for 5 min at room temperature (23 - 25°C). The mixture was centrifuged for 10 min at 12,000xg and the supernatant, which contained the enzymes, was heated for 15 min at 70°C to inactivate β-amylases. The heated supernatant was centrifuged (10 min at 12,000xg) and the supernatant used directly in the isoelectric focusing separation of the isoenzymes.

b. Isoelectric focusing. The α-amylases were separated by a gel electrofocusing procedure similar to that of Wrigley (1968). The procedure that was used is as follows. The gel solution (2.0 ml) comprised 0.4 ml of 50% sucrose solution, 0.1 ml of 2% Temed solution, 0.5 ml of 0.004% riboflavin solution, 0.75 ml of acrylamide solution (30 g acrylamide and 0.8 g bisacrylamide per 100 ml), 0.2 ml of sample and 0.05 ml of LKB ampholyte (pH 3.5 - 10). The gel solution was mixed, poured into the glass tubes (7.1 cm in length with inside diameter of 0.5 cm) and photopolymerized. Electrofocusing, in an E-C

disc-gel electrophoresis apparatus, was for 20 hr at 20 volts per tube in the cold room (4 - 8°C). The anodic and cathodic electrode solutions were 0.2M acetic acid and 0.2M ethylenediamine, respectively.

c. Detection of isoenzymes. The isoenzymes were localized by their degradation of β-limit dextrin immobilized in a separate polyacrylamide gel. The substrate gel solution was comprised of 8% acrylamide, 0.25% β-limit dextrin, 0.2M acetate buffer, pH 5.5 and 10^{-3}M $CaCl_2$. The solution was polymerized into a film between two glass plates separated by a 0.2 mm microscope slide cover. The gel containing the focused isoenzymes was placed in contact with the substrate film and incubated for 15 min at 37°C. Gels that contained high α-amylase activity (germinated samples) required only a 5-min incubation period. Following incubation, the substrate film was stained with a 0.4% potassium iodide 0.04% iodine solution. The areas that were in contact with α-amylase isoenzymes appeared as clear bands on a pink background.

3. Proteolytic Activity

a. Extraction. A 0.5 g sample of ground grain was extracted with 20 ml of 0.2M acetate buffer, pH 3.8 (Wang and Grant, 1969) by stirring with a magnetic stirrer for 1 hr at 4°C. The extract was centrifuged for 10 min at 10,000xg and filtered through a Whatman No. 40 filter paper. The filtrate was used directly for the assay of proteolytic activity.

b. Assay. Measurement of proteolytic activity was by a modified Ayre-Anderson method (Ayre and Anderson, 1939). The procedure used was as follows: Five ml of a 1% hemoglobin solution (in 0.2M acetate

buffer, pH 3.8) was added to 5.0 ml of the enzyme extract in a 50 ml centrifuge tube, tightly capped, and incubated for 20 hours with agitation at 37°C. After digestion, 10 ml of 5% trichloroacetic acid (TCA) solution was added to the digestion medium, centrifuged for 10 min at 10,000xg and filtered through a Whatman No. 40 filter paper. The absorbance of the filtrate was read at 280 nm against a blank on a Zeiss PMQ II Spectrophotometer. The blank contained all the assay components. However, the substrate (hemoglobin) solution was incubated alone and the enzyme extract was added to it after the addition of the TCA solution. Proteolytic activity was expressed in µmoles of tyrosine released per minute. The tyrosine concentration was read off a calibration curve prepared using pure tyrosine. One µmole/min of tyrosine released was taken as equivalent to one proteolytic unit (PU) of activity.

The possibility of bacterial growth during the long incubation period used in the protease assay was tested by the addition of 0.05% toluene or sodium azide to the assay medium. There was no significant decrease in activity.

4. Proteolytic Isoenzymes

a. Extraction. The extract for detection of protease isoenzymes was prepared as follows. A 0.5 g sample was added to 2.5 ml of 0.2M acetate buffer, pH 3.8, homogenized for 5 min in an ice bath and centrifuged for 15 min at 15,000xg. Sucrose (10%) was added to the supernatant to increase solution density and methyl green (trace) was added to act as visual marker. This solution was used directly for the

electrophoretic separation of the isoenzymes.

b. Electrophoresis. Proteolytic isoenzymes were separated by electrophoresis using an acidic system (0.017M aluminum lactate-lactic acid buffer, pH 3.0). Electrophoresis was performed in an E-C vertical slab gel apparatus using a 15% acrylamide gel (Andary and Dabich, 1974). The gel was polymerized by the ascorbic acid-ferrous sulfate-hydrogen peroxide catalyst system of Jordan and Raymond (1969). Gel solutions were cooled to approximately 0 to 2°C before the addition of hydrogen peroxide to slow down the rate of polymerization. The gel slab was pre-run for 30 min before the addition of enzyme solution.

c. Detection of isoenzymes. Immediately after electrophoresis, the gel was soaked in a 5% hemoglobin solution (in acetate buffer, pH 3.8) for 30 min, rinsed in acetate buffer (excess buffer removed from gel surfaces by wiping carefully with cellulose tissue) and incubated in a closed container (under moist atmosphere) for 5 hr at 37°C. After the incubation, the gel was soaked in 10% acetic acid-10% isopropanol solution containing 2% TCA for 40 minutes and stained with 0.01% Coomassie Brilliant Blue (CBB) solution (containing 10% acetic acid-10% isopropanol). Gel was destained with 10% acetic acid-10% isoproponol solution to remove excess dye. The proteolytically active bands (isoenzymes) appeared as white or clear bands on a blue background.

5. Peroxidase Activity

a. Extraction. The extract for peroxidase activity was prepared as follows. The ground grain sample (0.5 g) was extracted with 2.5 ml of 12.5% sucrose solution (LaBerge *et al.*, 1973) in a 15 ml centrifuge

tube by rotating for 1 hr on a Multi-Purpose rotator at room temperature (23 - 25°C). The mixture was centrifuged for 15 min at 20,000xg and filtered through Whatman No. 1 filter paper. The filtrate was used directly in the assay and a portion of the enzyme extract was saved and stored frozen for the isoenzyme analysis.

b. Assay. Peroxidase activity was determined by a method similar to that of Honold and Stahmann (1968). The assay mixture consisted of 3.8 ml of distilled water, 1.0 ml of 0.1M phosphate buffer pH 6.5, 1.0 ml of 0.1% O-dianisidine (Kruger and LaBerge, 1974), 0.2 ml of enzyme extract and 1.0 ml of 0.02% hydrogen peroxide solution. Hydrogen peroxide was added last to initiate the reaction. The reaction was monitored at 500 nm for 1 min at 25°C on a Cary 15 Spectrophotometer. Activity was expressed in peroxidase units (PU). One PU is the change in absorbance per minute under the assay conditions.

6. Peroxidase Isoenzymes

a. Electrophoresis. Anionic peroxidases were separated by electrophoresis at pH 8.9 according to Davis (1964). A 100 µl aliquot of extract (as in section 5a) was applied to each tube. Bromophenol blue was added to each tube as the electrophoretic marker dye. Cationic peroxidase isoenzymes were also separated by the same method except the electrodes were reversed. For the cationic peroxidases only a 10 µl sample was applied to each tube and crystal violet was used as the marker dye.

b. Detection of isoenzymes. Peroxidase isoenzymes were detected by a procedure based on the methods of Feillet and Kobrebel (1974) and

LaBerge *et al.* (1973). The procedure was as follows. After electrophresis the gels were soaked in a 1% aluminum lactate solution for 10 min with agitation. They were then transferred into a freshly prepared solution composed of 1 part of 0.1% O-dianisidine solution (in water) and 1 part 0.156% catechol solution (in a solution containing 1.95 g Tris, 0.2 g disodium ethylenediamine tetracetate, 0.15 g boric acid and 10 g calcium chloride per liter) and incubated for 30 min with agitation at room temperature (23 - 25°C). The O-dianisidine-catechol staining solution was then poured off and gels were incubated in a 0.02% hydrogen peroxide solution for 30 min with agitation. The peroxidase isoenzymes appeared as dark brown-black bands and were stable for several months when stored in a 10% acetic acid solution.

D. Proteins

1. Gel Filtration Chromatography

For gel filtration chromatography, the grain protein was extracted with AUC solvent (aqueous solution containing 0.1M acetic acid, 3M urea and 0.01M cetyltrimethylammonium bromide) of Meredith and Wren (1966). A 0.5-g sample of ground grain was homogenized with 4.0 ml AUC solvent for 10 min. The suspension was centrifuged for 10 min at 20,000xg and the residue was similarly extracted 2 more times with 3.0 ml of AUC solvent. The three supernatants were combined and filtered through Whatman No. 1 filter paper. Three ml of the filtrate was applied to a 2.5 cm x 50 cm Sephadex G-150 column (equilibrated with AUC) and eluted with AUC solvent. Two to three ml fractions were collected at 10-minute intervals. The elution volume and absorbance at 280 nm

(on Zeiss PMQ II Spectrophotometer) was recorded for each fraction. Elution profiles were obtained by plotting absorbance against elution volume.

2. SDS-PAGE of AUC Extracts

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at pH 8.9 was performed in an E-C vertical slab gel apparatus according to Koenig *et al.* (1970), using eight slots per gel. Ten mg of the freeze-dried AUC-protein extract was dissolved in 1 ml of protein solvent (2% SDS in 0.125M Tris-Borate, pH 8.9), with and without β-mercaptoethanol (1%). The sample was centrifuged for 10 min at 40,000xg to remove a minute quantity of insoluble material. Approximately 10% sucrose was added to increase density and a trace of bromophenol blue was added as the tracking dye. The gel was pre-run for 30 min after polymerization. Protein solutions (100 µl) were applied to gel slots and electrophoresis was performed at 200 volts (105 to 110 ma) until the tracking dye moved 8 cm. The gels were then soaked in 10% TCA solution for 30 min stained with 0.05% CBB solution of acetic acid-ethanol-water (1:4.5:4.5) for 8-12 hr, and destained with acetic acid-methanol-water (1:2.5:9) solution for 2 to 3 days. The molecular weights of the components of the reduced and unreduced AUC-soluble proteins were estimated by comparing the mobilities with those of standard proteins under the same conditions (reduced and unreduced).

3. Solubility Fractionation

The proteins of ground grain samples were fractionated according to solubility in various solvents that were essentially the same as those

used in the classical method of Osborne (1907). The actual procedure used was as follows. One g of ground grain (or flour) was extracted three times (2 x 10 min, 1 x 5 min) with 5.0 ml of 0.5M sodium chloride solution in an ice bath by homogenizing in a 15 ml centrifuge tube with a specially fitted plexiglass plunger. Each extraction was followed by a 10 min centrifugation at 20,000xg. The residue was extracted with 5.0 ml of distilled water for 5 min to remove residual salt. The four supernatants were combined and the volume measured accurately.

The salt-soluble fraction was also used to determine non-protein nitrogen content of the various samples studied. The non-protein nitrogen content was that portion of the salt-soluble extract that was soluble in 2.5% TCA solution.

The residue was extracted (as in the case of the salt-soluble fraction) sequentially three times with 70% ethanol and the supernatants were combined. The residue from the alcohol extraction was extracted similarly with 0.05M acetic acid and the residue from this extraction was extracted with 0.1M sodium hydroxide solution. After the volumes of the fractions were recorded, they were filtered through glass wool. The final residue was suspended in the sodium hydroxide solution. Nitrogen content of each fraction was determined by the micro Kjeldahl procedure in duplicate on 2.0-ml aliquots. Ammonium sulfate was used to calibrate the Kjeldahl procedure. The remainder of each fraction was dialyzed against distilled water and freeze-dried. The dry proteins were used in the electrophoresis analysis.

4. Electrophoresis of Protein Fractions

Electrophoresis was performed in an Ortec model 4200 electrophoresis apparatus using an acidic discontinuous system on polyacrylamide gel. Table 2 gives the composition of the upper and lower gels used. Upper and lower gels were photopolymerized for 15 and 10 min, respectively. For electrophoresis, the protein fractions were dissolved according to Chen and Bushuk (1970B) using the appropriate solvents for each fraction. For the alkali-soluble fraction, the solvent was the same as that used for the acetic acid-soluble fraction (4M N,N-dimethylformamide-0.1M acetic acid solution). The volumes applied to the gels were 100 µl for the salt-soluble fraction and 50 µl for the other three fractions. Stock buffer solutions for upper and lower electrolytes were made up as follows: upper buffer (pH 4.0), 2.5 ml of acetic acid and 28.1 g of glycine per liter; and lower buffer (pH 4.3), 23.0 ml of acetic acid and 120 ml of 1N potassium hydroxide per liter. The stock buffer solutions were diluted to 1/10th of original concentration just prior to use. Electrophoresis was performed in the cold room (4 - 6^{o}C) at a constant voltage of 400 volts, using two gels per run. The current was held at 65 ma for the first 15 min, then increased to 130 ma for the remainder of the electrophoresis period. Electrophoresis was performed for both 60 and 180 minutes. After completion of the electrophoresis, the gels were soaked in a 10% TCA solution for 20 min and then stained with a 0.05% nigrosine solution containing acetic acid-ethanol-water (1:4.5:4.5) for approximately 10 - 12 hr. The gels were destained by soaking in a solution comprising acetic acid-methanol-water (1:2.5:9) for approximately 24 - 48 hr. For permanent records the gels were photographed on Panatomic-X film.

TABLE 2. COMPOSITION OF SOLUTIONS FOR DISCONTINUOUS GEL ELECTROPHORESIS.

Chemicals	Upper Gel (pH 5.8) Per 100 ml	Lower Gel (pH 2.9) Per 100 ml
SOLUTION A[1]		
Acrylamide (g)	10.0	30.0
N,N-methylene-bisacrylamide (g)	0.8	0.8
SOLUTION B[1]		
In potassium hydroxide (ml)	29.0	6.0
Glacial acetic acid (ml)	1.7	26.5
N,N,N,N-tetra-methylenediamine (ml)	0.24	0.48
SOLUTION C[1]		
Ammonium persulfate (mg)	120	120
Riboflavin (mg)	2	2
SOLUTION D[1,2]		
Sodium sulfite (mg)	40	40

[1] Gel solution consists of equal volumes of solutions A, B, C and D.

[2] Freshly prepared.

V. RESULTS AND DISCUSSION

A. Physical Characteristics, Moisture and Nitrogen Contents

1. Expression of Results

Comparative data for grain at different stages of maturity can be expressed on a kernel, dry weight, or as is percentage basis. Each method of expression has advantages and disadvantages. In the present investigation, expression of results on a kernel basis proved unsatisfactory because of the large differences in kernel size and weight among the various cereals that were examined in this investigation. (Table 3 illustrates these differences for six cereals at maturity.) To eliminate this difficulty the results on a kernel basis were normalized by dividing each value by the mature kernel volume (in cm^3). Results expressed in this manner are similar to the results on a kernel basis, except that differences due to kernel size are eliminated by expressing data on per unit kernel volume.

The development of the various grain constituents during grain maturation yield different patterns when data are represented on a per kernel per cm^3 or on a weight concentration basis. Large differences among cereals and grain of different degree of maturity within a species are detectable when either method of expressing the results is used. The results in the text of this thesis will be given using both methods of expressing results.

TABLE 3. PHYSICAL CHARACTERISTICS OF MATURE GRAINS.

Cereal Species	Kernel Weight (mg)	Kernel Volume (cm^3)	Kernel Density (g/cm^3)
Triticale 6A190	49.2	0.049	1.003
Triticale 6517	38.7	0.036	1.068
Triticale 6A250	36.6	0.032	1.143
HRS wheat	34.1	0.028	1.219
Durum wheat	52.3	0.043	1.230
Spring rye	33.1	0.029	1.131

2. Physical Characteristics

Selection of the three triticales which exhibit definite differences in the degree of kernel shrivelling at maturity were based entirely on visual assessment. However, Klassen *et al.* (1971) demonstrated that grain density could be employed to measure the severity of this physical defect (see Table 3). The absolute density values are somewhat different, but the relative order of the values for the six cereals is the same as that obtained earlier by Klassen *et al.* (1971). Stewart 63 and Manitou wheats had the highest densities. The most shrivelled triticale, 6A190 had the lowest density, considerably lower than the density of either of its parents, Prolific rye or Stewart 63 durum. For the three triticales used in this investigation, the increase in degree of grain shrivelling parallels the decrease in grain density, in agreement with the findings of Klassen *et al.* (1971).

The grains used in this investigation showed a wide range in kernel size and weight (see Table 3). Changes in dry matter content (freeze-dried weight) with kernel development are given in Table 4. All cereals showed a gradual increase in kernel dry matter with development. The increase is essentially linear during development (from 12 to 26 days) and then levels off as the grain approaches maturity. Kernel weights of the various cereals at any particular stage of development differs quite widely; the order parallels that of kernel weight at maturity. The changes observed in this study are similar to those of Klassen (1970) for eight triticale strains (lines) used in his study.

TABLE 4. DRY MATTER CONTENT AT DIFFERENT STAGES OF KERNEL DEVELOPMENT.

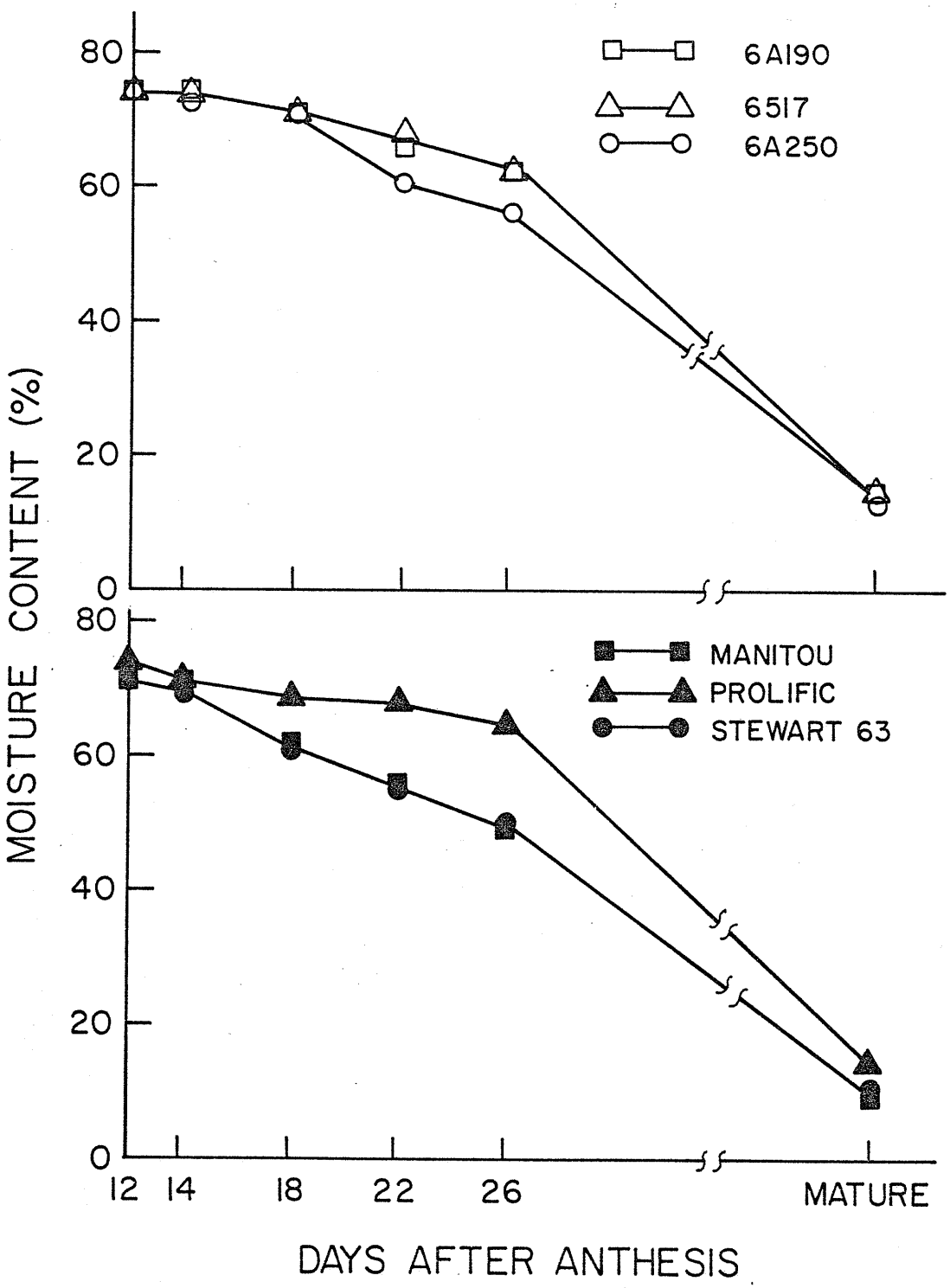
Cereal Species	Kernel Weight (mg)				
	Days after Anthesis				
	12	14	18	22	26
Triticale 6A190	10.0	12.4	22.3	30.0	37.6
Triticale 6517	8.4	11.1	17.2	23.9	29.4
Triticale 6A250	7.9	10.2	16.9	23.6	29.2
HRS wheat	6.8	9.2	14.8	19.4	24.1
Durum wheat	9.2	13.5	21.7	28.7	34.7
Spring rye	5.4	6.5	10.2	15.4	19.5

On the other hand, Jenkins and Meredith (1975) observed that during a short period at near ripeness triticale and wheat showed a slight decrease in dry-matter content. Subsequently, triticale not only regained this lost weight but continued to gain weight for at least a month after conventional ripeness. The gain in dry matter in the post-ripe stage was observed in the study by Klassen (1970) for some triticale strains, but was not observed in the present study due to insufficient sampling periods at this stage of grain development.

3. Moisture Content

As expected, the moisture content decreased with maturation for all cereals (Figure 2). Initial (12 days after anthesis) values ranged from 71.3 to 74.5% and decreased with maturation to 9.4 to 15.0% at maturity. Distinct differences were apparent among the cereals at different stages of kernel development. The two wheats had similar moisture contents, but were significantly lower than the values for rye or the three triticales at all stages of kernel growth. Klassen (1970) found that rye and triticale retained moisture for a longer period during development than the wheats. Differences in moisture content were most evident at 22 and 26 days after anthesis stage, not only among the various cereals species, but also among the three triticale strains. The shrivelled and plump triticales have respectively 11.4 and 5.3% higher moisture contents (at 22 and 26 days after anthesis stage) than the wheats. Jennings and Morton (1963) found that in maturing wheat, the rapid loss of water (corresponds to the initiation of the mature phase) was not only dependent on variety

Figure 2. Moisture content at various stages of kernel development.


80
60
40
20
0
6A190
6517
6A250
MANITOU
PROLIFIC
STEWART 63
MOISTURE CONTENT (%)
12
14
18
22
26
MATURE
DAYS AFTER ANTHESIS

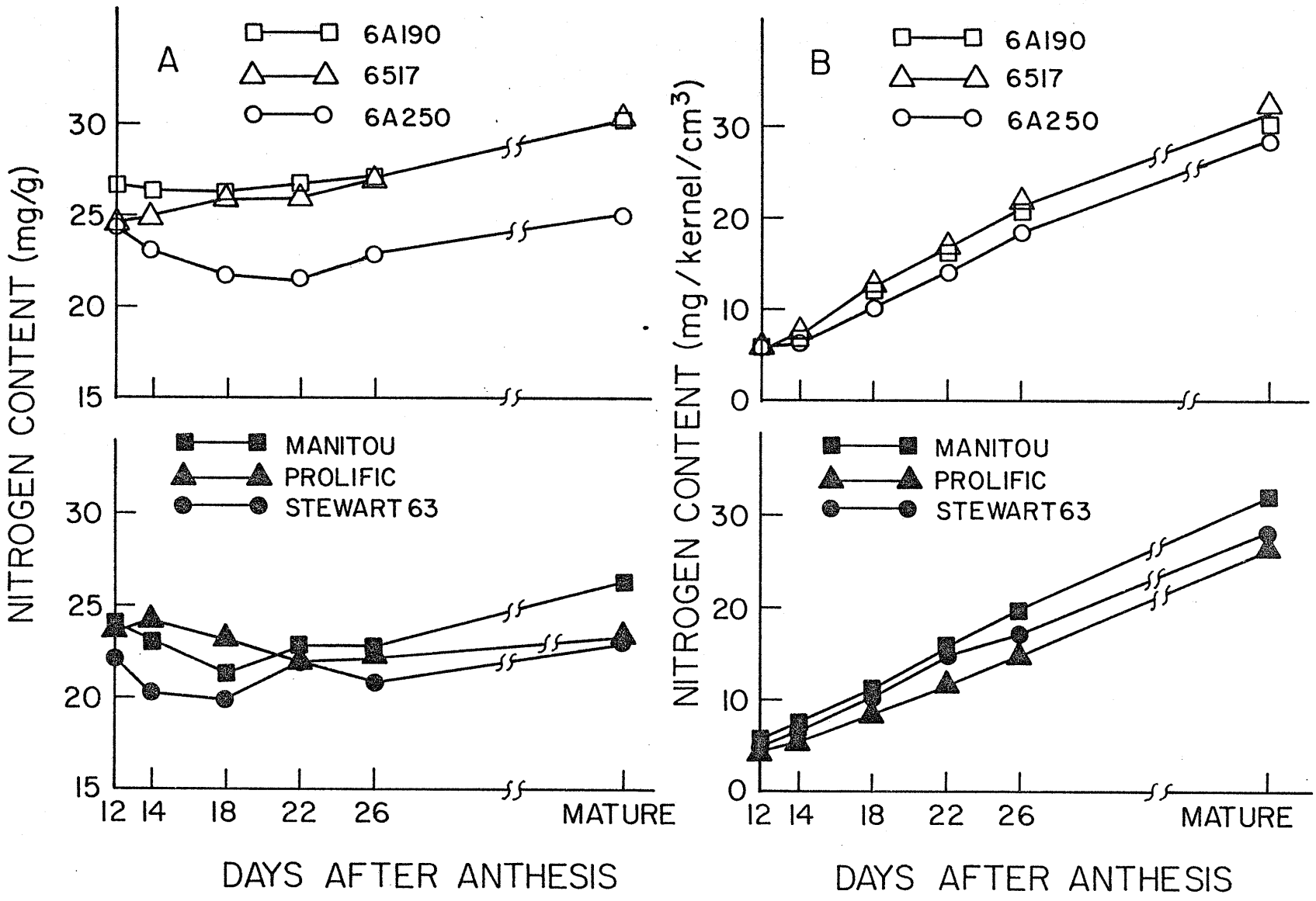
but also on the availability of moisture in the soil (rainfall). Since the six cereals in the present investigation were grown under identical field conditions, the observed differences in moisture content are most likely due to varietal (and species) differences rather than to environmental factors. Because of the wide variation in moisture content among the species investigated the days after anthesis was used as an index of grain maturity rather than moisture content (as used by some workers). However, the author is cognizant of the fact that differences in moisture content may reflect differences in physiological maturity.

4. Total Nitrogen Content

Figure 3 shows the total nitrogen content at various stages of grain development for each cereal investigated. In the two triticale strains that produce highly shrivelled grain, the total grain nitrogen content (Fig. 3A) increased slowly throughout the growth period. The strain 6A190 (highest in degree of shrivelling) had a slightly higher nitrogen content than the strain 6517 (second in degree of shrivelling) in the four earliest samples. The triticale that yields plump grain and the other three cereals showed a decrease in total nitrogen from 12 to about 22 days after anthesis followed by a gradual increase to maturity. Similar results were obtained for wheat by Jennings and Morton (1963). This is considered to be the normal development pattern for cereal grains.

Figure 3 also shows that the two triticales that yield shrivelled grain contain considerably more nitrogen at all stages of development than the other four species examined. At 18 days after anthesis and

Figure 3. Total nitrogen content at various stages of kernel development.


A
B
6A190
6517
6A250
MANITOU
PROLIFIC
STEWART 63
NITROGEN CONTENT (mg/g)
NITROGEN CONTENT (mg/kernel/cm3)
30
25
20
15
10
0
12 14 18 22 26 MATURE
DAYS AFTER ANTHESIS

thereafter, the two shrivelled triticales contained approximately 4.3 mg per gram more nitrogen than the plump triticale (6A250). The results obtained for triticale 6A190 and its parents are in general agreement with the findings of Dexter (1974), except that in this previous study no increase in nitrogen content was observed for Prolific rye at maturity over the values at earlier stages of development.

When the results were expressed on a per kernel per cm^3 basis (Fig. 3B) all six cereals showed an almost linear increase in total grain nitrogen during early grain development. Towards the later stages of maturation, the amount of total kernel nitrogen tended to level off. These results are similar to those obtained for wheat (Jennings and Morton, 1963), barley (MacGregor *et al.*, 1971), triticale 6A190 and its parental species (Dexter and Dronzek, 1975A). As found by Dexter and Dronzek (1975A), the present study showed that the rye had the lowest nitrogen content at all stages of growth, while the shrivelled triticales had the highest values. Past 14 days after anthesis, triticale 6A250 grain had a significantly lower nitrogen content than the grain of the two shrivelled varieties.

The differences in total nitrogen content among maturing triticales that differ in degree of grain shrivelling may be explained two ways. First, the difference may be a true characteristic of this physical defect; and secondly, it may be due to depressed synthesis of other grain constituents such as starch. This would result in higher than normal nitrogen content values on a weight basis. Since

the amount of nitrogen (presumably protein) on a per kernel per cm^3 basis increased with maturation and no decrease was observed on a percentage basis (Fig. 3), this would suggest that the second of the above explanations for the increase in total nitrogen is more plausible.

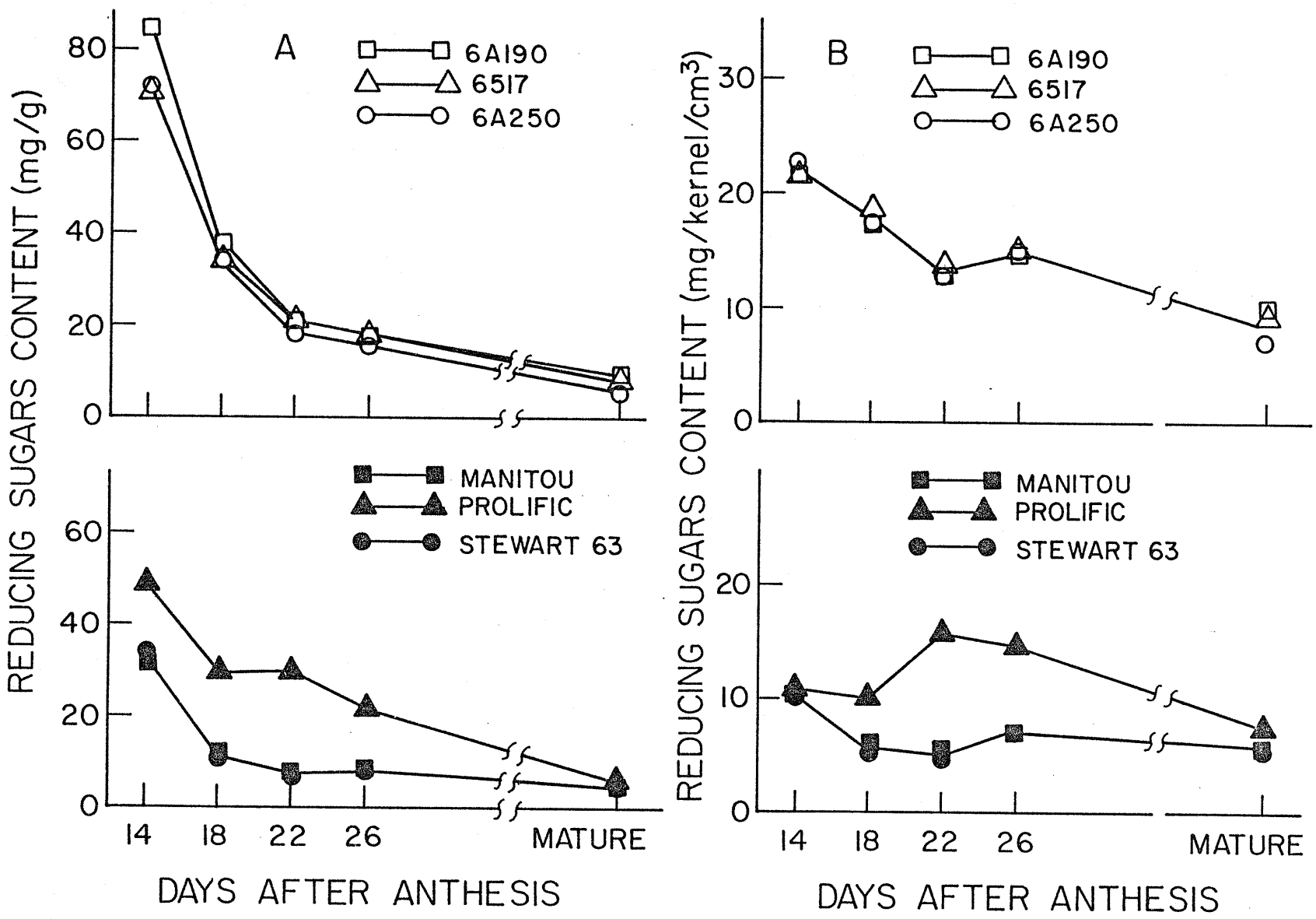
B. Carbohydrates

1. Reducing Sugars

In all species investigated reducing sugars content of the grain decreased, rapidly at first and then more slowly, as the grain developed and matured (Fig. 4A). These results are in general agreement with published results for maturing wheat (Abou-Guendia and D'Appolonia, 1972) and barley (LaBerge *et al.*, 1973). The present study showed that large differences exist among the three classes of cereals in the absolute amounts of reducing sugars present in grain of specified maturity. For example, at 14 days after anthesis the three triticale lines had the highest reducing sugars content (from 70.6 to 84.7 mg per gram of sample), the content of rye (48.5 mg) was intermediate and the HRS and durum wheat (30.7 mg and 33.9 mg respectively) were the lowest. The reducing sugars contents of the three triticales were essentially the same at all stages of maturity. Accordingly triticale lines that yield grain with different degrees of shrivelling cannot be distinguished on the basis of reducing sugars content.

When the reducing sugars content was expressed on a per kernel per cm^3 basis (Fig. 4B) the trends were similar to those shown in Fig. 4A, except that by this method of plotting the data, the values

Figure 4. Changes in reducing sugars content during kernel development.


A
B
6A190
6517
6A250
MANITOU
PROLIFIC
STEWART 63
REDUCING SUGARS CONTENT (mg/g)
REDUCING SUGARS CONTENT (mg/kernel/cm3)
0
20
40
60
80
10
30
14
18
22
26
MATURE
DAYS AFTER ANTHESIS

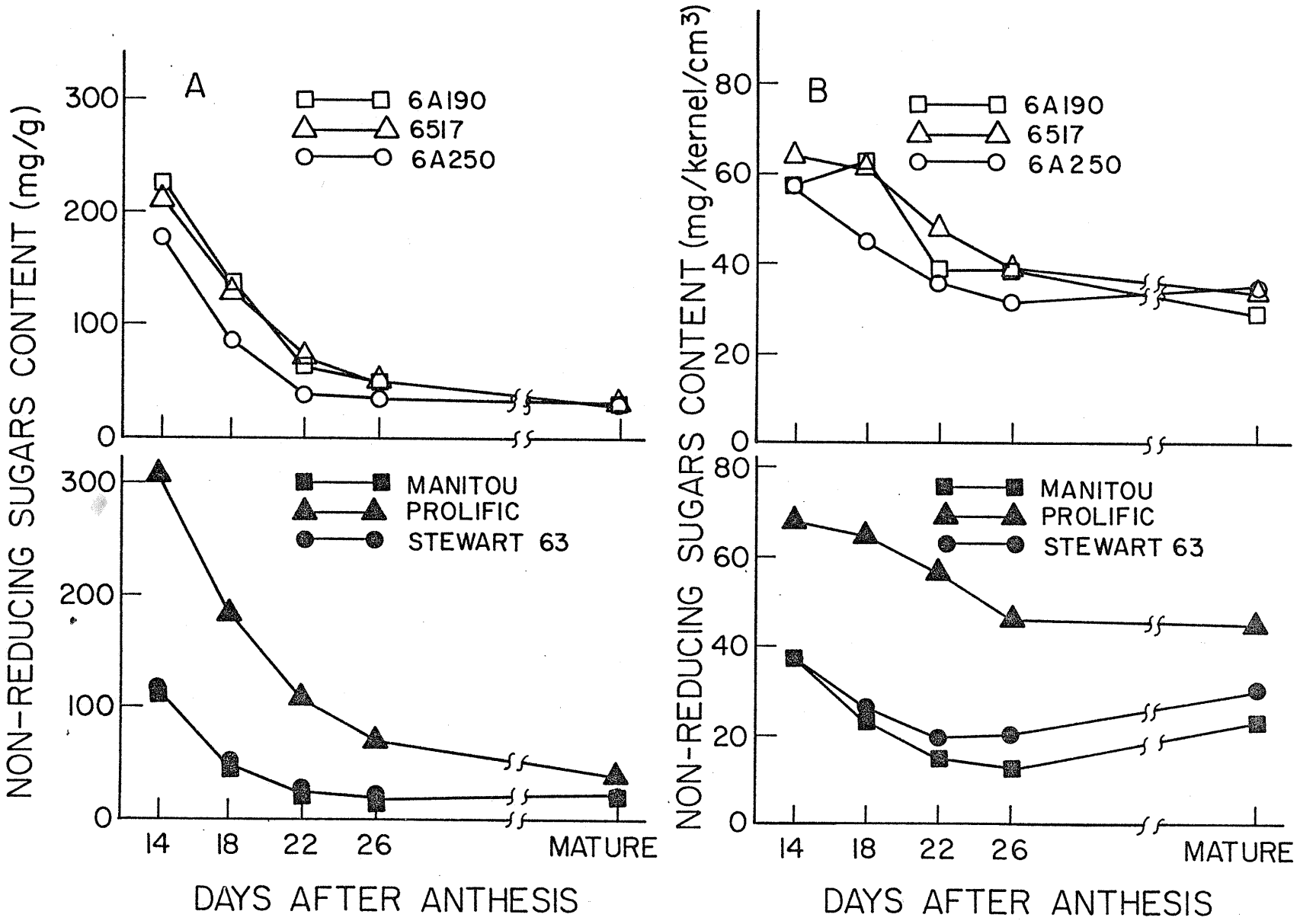
for rye in the 22- and 26-day samples were higher than those for the 14- and 18-day samples. The triticales and the wheat samples showed some indication of this trend also (the 26-day values were somewhat higher than the 22-day values). This rise in reducing sugars content appears to coincide with rapid synthesis of starch (to be discussed later).

The data of Fig. 4B showed similar trends to those obtained by Jennings and Morton (1963) and Klassen *et al.* (1971) for maturing wheat and triticale, respectively. The curves for the three triticales investigated in the present study are almost identical. Reducing sugars content of mature grain was slightly higher for the two lines that produce shrivelled grain than for the line that yields plump grain. However, the difference is not considered significant. Klassen (1970) observed an increase in reducing sugars content in several triticale lines in the late stages of maturation; this was not confirmed by the present study. The reason for this discrepancy was that in the present investigation grain was harvested only at maturity, after the 26-day stage of grain development, while in the previous study grain was collected at more regular intervals after the same stage of development.

2. Non-Reducing Sugars

The non-reducing sugars content of the grain (Fig. 5A) showed a similar decreasing pattern as observed for the reducing sugars. The trend observed for all six cereals was similar to that obtained for wheat by Jennings and Morton (1963) and Abou-Guendia and D'Appolonia (1972).

Figure 5. Changes in non-reducing sugars content during kernel development.


A
B
6A190
6517
6A250
MANITOU
PROLIFIC
STEWART 63
NON-REDUCING SUGARS CONTENT (mg/g)
NON-REDUCING SUGARS CONTENT (mg/kernel/cm³)
0
100
200
300
20
40
60
80
14
18
22
26
MATURE
DAYS AFTER ANTHESIS

The three classes of cereals were characterized by markedly different non-reducing sugars content, especially in the grain at early stages of development. Prolific rye had the highest non-reducing sugars content at all stages of development, followed by the three triticales and then the two wheats which had the lowest contents. In 14-day grain, the non-reducing sugars comprised approximately 10%, 20% and 30% of the dry weight for the wheats, the triticales and the rye, respectively. In mature grain, the equivalent amounts were 2%, 3% and 4%.

The non-reducing sugars contents for the shrivelled triticale lines were higher than the values for the plump-grained strain for immature grain. The values for mature grain were essentially identical. Accordingly, it would appear that shrivelled- and plump-grained triticale lines can be differentiated on the basis of non-reducing sugars content of immature grain but such a distinction would not be possible on the basis of analyses of mature grain. Since the differences are at a time of rapid kernel growth, it may be difficult to select grains from different strains (which would differ in "day to maturity") of equal physiological maturity.

When the non-reducing sugars content was expressed on a per kernel per cm^3 basis (Fig. 5B) the trends and the relative order of the cereals in absolute content was the same as that inferred from Fig. 5A, except that the values for the mature wheat were slightly higher than the values for the 26-day grain. The results obtained in the present study are in general agreement with those of Abou-Guendia and D'Appolonia (1972) who found that non-reducing sugars content of their

wheats showed some fluctuation with grain maturation, but an overall decreasing trend was quite apparent.

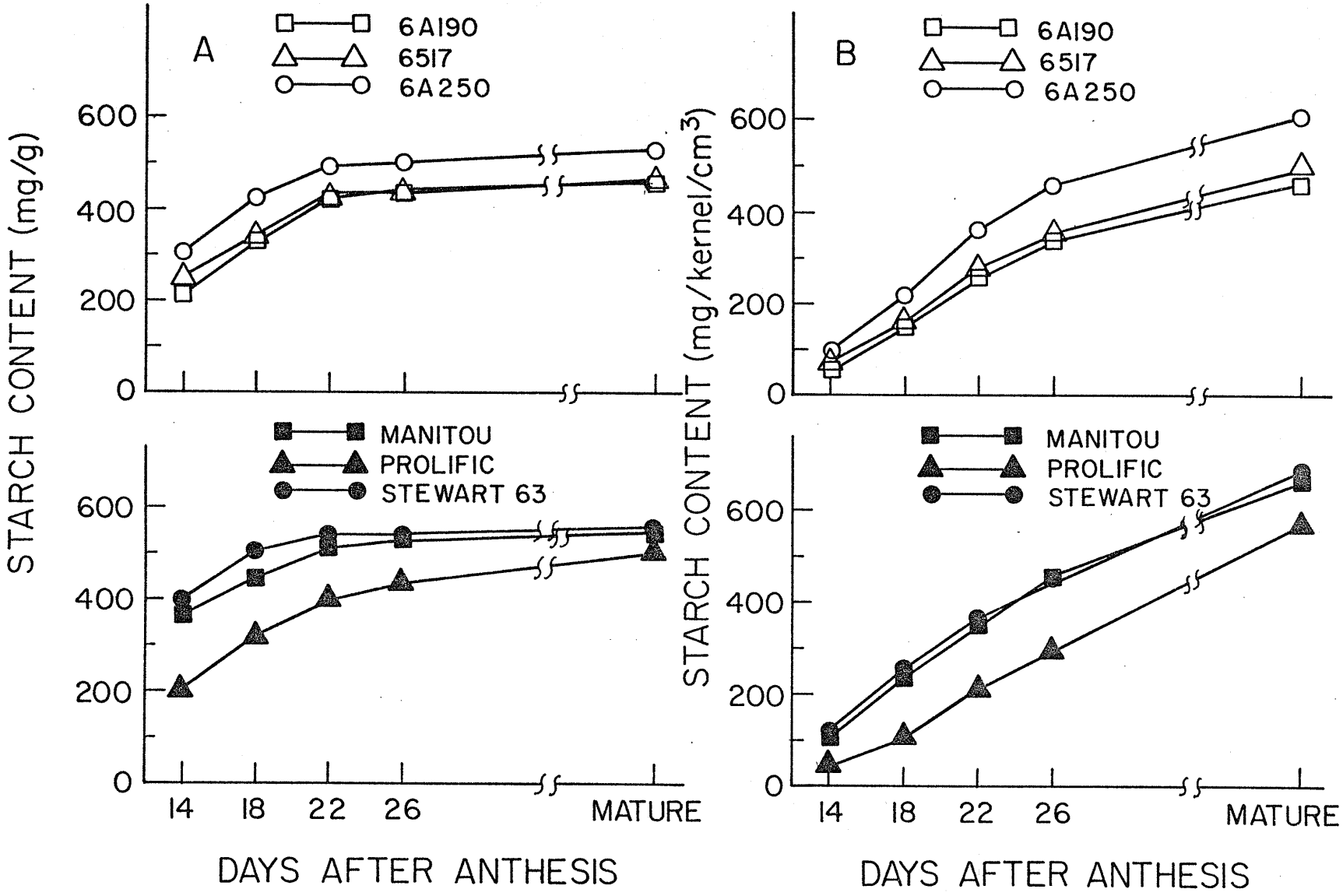
The reducing sugars levels obtained in the present investigation are three to four times higher than those of Klassen *et al.* (1971) for the same triticale strains. The reason for the discrepancy between these values was not investigated. The results of the present investigation agree with the findings of Vaisey and Unrau (1964) who showed that in flour of mature cereal grain the non-reducing sugars fraction constitutes the largest portion of the total soluble sugars.

3. Starch Content

The starch content increase rapidly during early development (14-26 days after anthesis) and then remained essentially constant to maturity (Fig. 6A). Rye had the lowest starch content, the three triticale lines had intermediate starch content and the two wheats had the highest contents. The relative values for the six cereals were the same when results were expressed in mg of starch per kernel per cm^3. The starch development patterns during grain growth for these cereals are in general agreement with the patterns obtained by Jennings and Morton (1963) and Abou-Guendia and D'Appolonia (1973) for wheat.

Of the two wheats the durum cultivar had slightly higher starch values than the HRS wheat cultivar. Starch synthesis and deposition in rye and the two triticales that yield shrivelled grain were similar

Figure 6. Starch content of various stages of kernel development.


A
B
6A190
6517
6A250
MANITOU
PROLIFIC
STEWART 63
STARCH CONTENT (mg/g)
STARCH CONTENT (mg/kernel/cm³)
0
200
400
600
14
18
22
26
MATURE
DAYS AFTER ANTHESIS

during early kernel development; at maturity rye had approximately 10% more starch. The percentage of starch in the plump-grained triticale (6A250) was slightly lower than the values for the HRS wheat, but was significantly higher, at equivalent stage of development, than the values for the two shrivelled triticale lines. The plump-grained triticale line had approximately 60 mg of starch per gram of sample more than the shrivelled grain of lines 6A190 and 6517. These findings are in general agreement with the results of Jennings and McCombs (1969) who showed that shrivelled maize kernels had considerably lower starch contents than the grain from plump-kernelled lines.

The rapid accumulation of starch at the early stages of development occur at a time when both reducing and non-reducing sugars contents decreased most rapidly. These relative changes in sugars and starch reflect a precursor-product relationship that exists between these substances. Similar indirect relationships were obtained for wheat by Jennings and Morton (1963) and Abou-Guendia and D'Appolonia (1973).

The curves for starch content, expressed on a per kernel per cm^3 basis (Fig. 6B) are essentially the same as those obtained by Klassen (1970) in his study of the relationship between starch content and grain density.

Klassen *et al.* (1971) suggested that starch accumulation ceases at an earlier stage during grain development in triticale than in wheat and that this was partly responsible for triticales' lower grain

densities. The amounts of starch accumulated between day 26 and maturity in the grain of the three triticales are 119.4, 147.4 and 149.1 mg per kernel per cm^3 for 6A190, 6517 and 6A250, respectively. These are considerably lower than the comparative values for hard red spring wheat, durum wheat and rye which are 211.4, 245.9 and 277.3 mg per kernel per cm^3, respectively. These results support the suggestion of Klassen *et al.* (1971) that starch accumulation in triticale grain stops at an earlier stage in kernel development than in wheat grain. This is most readily evident from comparison of the starch curves (Fig. 6B) for the HRS wheat and the plump triticale (6A250). During the early stages of development (from day 14 to day 26) the starch contents of these two species were almost identical, but at maturity, the value for the HRS wheat was 55.8 mg per kernel per cm^3 higher than for the plump triticale. This large difference in starch content between shrivelled and plump triticales is evident at early stages of kernel development. This suggests that the starch synthesizing apparatus in the shrivelled triticales is not functioning as well as in the plump triticale 6A250 from the beginning of starch synthesis. This may be the major reason for the lower starch contents in the shrivelled triticales. The higher sugars contents in the grain of shrivelled triticales is probably due to the fact that its incorporation into starch is depressed.

Thung (1970) observed that the amylose content of starch can be used to distinguish between smooth and wrinkled pea varieties. Accordingly, it was of interest to examine if a similar relationship exists

in triticale lines that yield grain with different degrees of shrivelling. The amylose contents, for grains of the six cereals used in this study, expressed in two different ways, are shown in Fig. 7. The trend in amylose content, with grain maturity, and the relative contents for the six cereals were the same as those obtained for starch content (discussed above). Analogous statements can be made about the amylose content to those made about the starch content.

Additional information on the status of the starch component in developing grain can be obtained by examining the trends in the ratio of amylose to amylopectin. Such data for the samples used in this study are tabulated in Table 5. This data was obtained from amylopectin values determined by subtracting the amylose content from the starch content. All cereals showed a gradual increase in the ratio of amylose to amylopectin during maturation. Similar results were obtained for wheat by Abou-Guendia and D'Appolonia (1973). The ratios for the plump triticale 6A250 increased from 0.114 at day 14 to 0.227 at the final stage of maturity. For the shrivelled triticales 6A190 and 6517, the ratio increased from 0.083 and 0.107 at day 14 to 0.196 and 0.182 at maturity, respectively. In samples harvested at 14 days after anthesis, the amylose to amylopectin ratio decreased with increasing degree of kernel shrivelling. This relationship was maintained for 18- and 22-day samples. The value for the 26-day sample of triticale 6517 appears to be too high, probably due to an error in analysis.

Figure 7. Amylose content at various stages of kernel development.

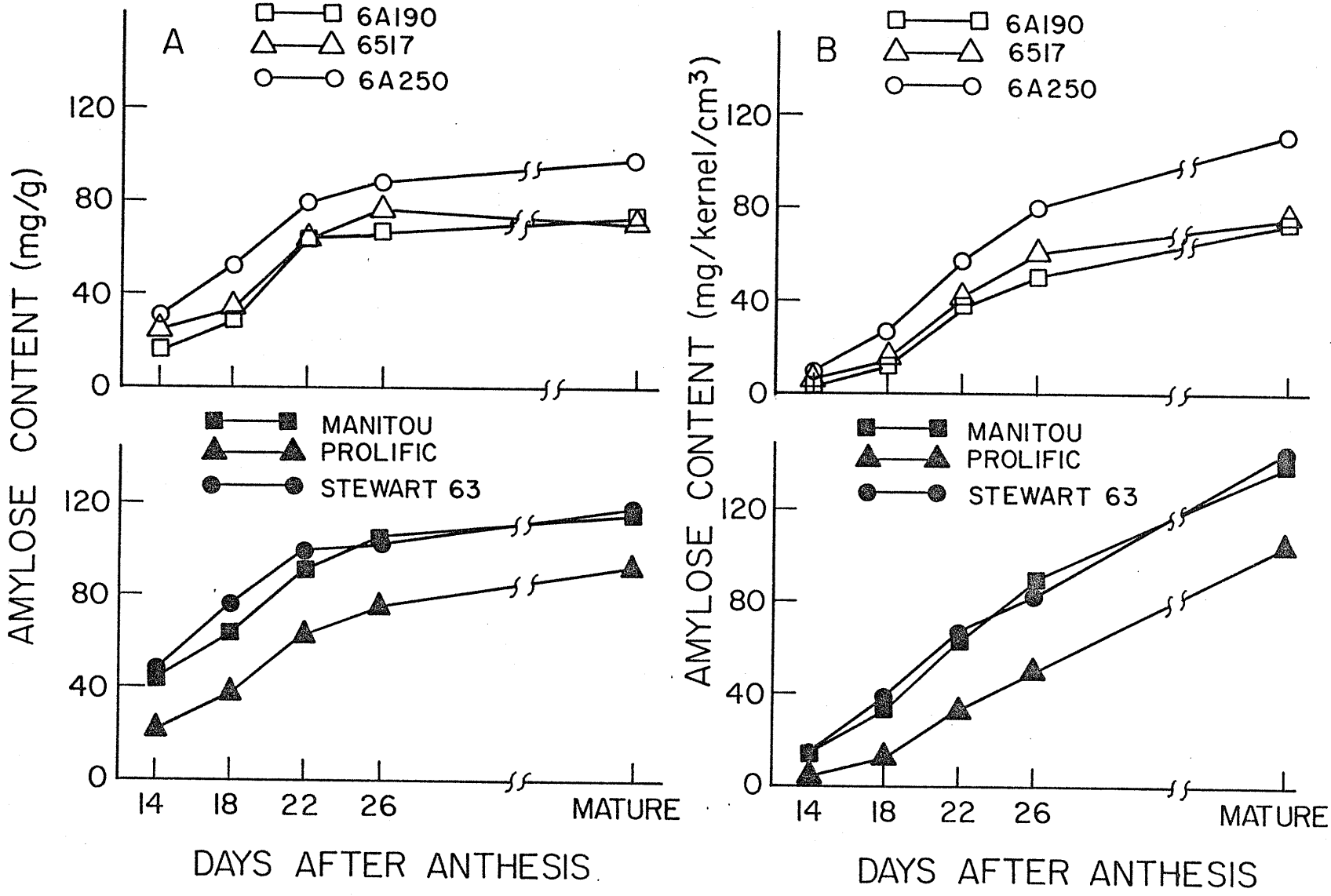

A
6A190
6517
6A250
MANITOU
PROLIFIC
STEWART 63
AMYLOSE CONTENT (mg/g)
0
40
80
120
14
18
22
26
MATURE
DAYS AFTER ANTHESIS
B
AMYLOSE CONTENT (mg/kernel/cm3)

TABLE 5. CHANGES IN THE AMYLOSE TO AMYLOPECTIN RATIO DURING KERNEL DEVELOPMENT.

Cereal Species	Amylose/Amylopectin Ratio				
	Days after Anthesis				
	14	18	22	26	Mature
Triticale 6A190	.083	.094	.178	.178	.196
Triticale 6517	.107	.112	.175	.212	.182
Triticale 6A250	.114	.141	.192	.212	.227
HRS wheat	.135	.166	.217	.244	.270
Durum wheat	.137	.178	.222	.238	.270
Spring rye	.120	.128	.188	.208	.227

The amylose/amylopectin ratios indicate that amylose synthesis is significantly lower in the shrivelled triticales than in the plump strain during the early stages of grain development and remains so through to maturity.

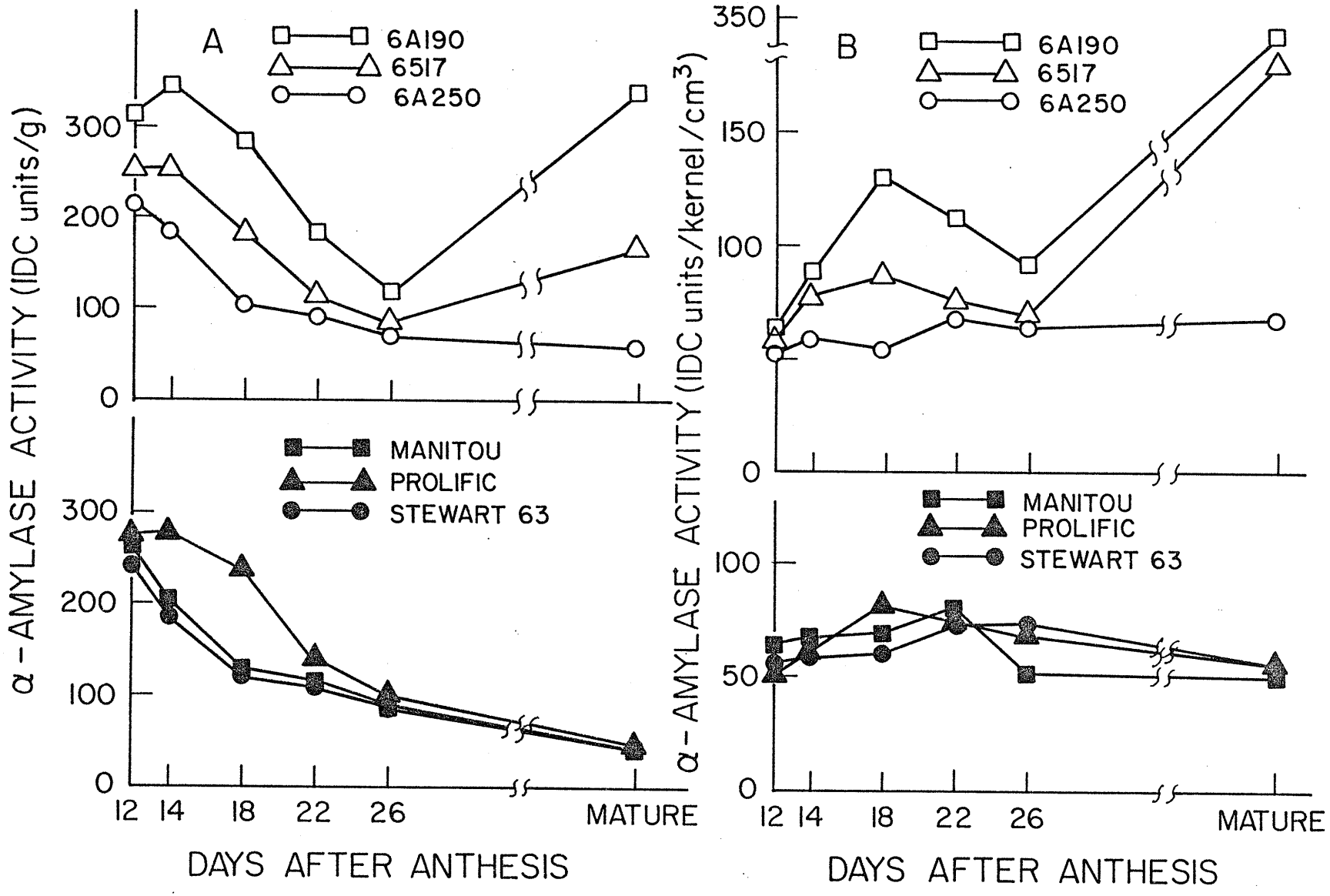
Of the six grains analyzed, the two wheats had the highest amylose/amylopectin ratios throughout the grain development period. The ratios for rye were similar to the values for triticale 6A250, the line that yields plump kernels. In the case of peas, Greenwood and Thomson (1962) found that wrinkled kernels had considerably lower starch content than the smooth kernels, but the amylose content of wrinkled peas was approximately two times higher than that of the smooth variety. In the triticale examined in the present study, both the starch and amylose contents were significantly higher in the plump line than in the two shrivelled lines. Obviously the involvement of starch in the shrivelling of the seed in the two plant species is quite different.

C. Enzymes

1. α-Amylase Activity

Klassen *et al.* (1971) found high correlation between the degree of kernel shrivelling and α-amylase activity in mature triticale. Accordingly, α-amylase activity was determined for the six cereals used in the present study for grain harvested at different stages of development. Figure 8A shows the activity of grain at different stages of maturity. In 12-day grain the activity was relatively high.

Figure 8. Variations in α-amylase activity during kernel development.


A
6A190
6517
6A250
MANITOU
PROLIFIC
STEWART 63
α-AMYLASE ACTIVITY (IDC units/g)
300
200
100
0
12 14 18 22 26 MATURE
DAYS AFTER ANTHESIS
B
6A190
6517
6A250
MANITOU
PROLIFIC
STEWART 63
α-AMYLASE ACTIVITY (IDC units/kernel/cm³)
350
150
100
50
0
12 14 18 22 26 MATURE
DAYS AFTER ANTHESIS

In the most shrivelled triticale (6A190) the value for the 14-day sample was higher than for the 12-day sample. Then there was a rapid decline in activity with maturation for all cereals. From 26-days after anthesis to maturity, the activity of the wheats, rye and the plump triticale declined slightly to the low level of activity in mature grain. On the other hand, in the two shrivelled triticale lines the activity increased sharply from 26-days to maturity. For all stages of development, the two wheats had the lowest activity, while the most shrivelled triticale (6A190) had the highest activity. For the early stages of development (12 to 26 days) the activity versus grain maturity curve for the plump triticale 6A250 is similar to the curves for the wheats, whereas the curves for the two shrivelled triticales resemble the curve for rye.

The two triticales that yield shrivelled grain were characterized by rapid increases in α-amylase activity from 26-days to maturity; the rate of increase was higher for the line with the highest degree of grain shrivelling. For mature grain, α-amylase activity (per gram of grain) was 340, 168 and 59 units for triticale 6A190, 6517 and 6A250, respectively. The order of activity was the same as the degree of shrivelling as assessed visually, with the most shrivelled grain having the highest activity. Increases in activity during the late stages of grain development of several different triticales were observed by Klassen *et al.* (1971) and Jenkins and Meredith (1975).

Somewhat different curves were obtained when α-amylase activity was expressed on a per kernel per cm^3 basis (Fig. 8B). The shape of

the curves for the two shrivelled triticale lines was similar to that of the analogous curves in Figure 8A. For the two wheats, the rye and triticale 6A250 the activity remained essentially constant throughout the development period. These results are in general agreement with the findings of Hill *et al.* (1973) and Jenkins and Meredith (1975) on α-amylase development in triticale grains expressed on per kernel basis. Both methods of plotting the data clearly distinguish the two shrivelled triticale lines from the plump line and show differences that can be related to the degree of shrivelling. However, it is not possible to say that either method is more fundamentally correct than the other. For this reason, the results of this study have been examined graphically using the two different units.

2. α-Amylase Isoenzymes

The increase in α-amylase activity in triticale grain, between 26 days after anthesis and maturity discussed earlier, paralleled the severity of kernel shrivelling; the line that showed the highest degree of shrivelling also showed the largest increase in α-amylase activity during this stage of maturation. The other cereal species, rye and wheat, did not show an increase in α-amylase activity during the same stage of grain development. The increase in α-amylase activity in one shrivelled triticale strain (6A190) may be due to premature germination (Hill *et al.*, 1973). LaBerge *et al.* (1971) observed a similar increase in α-amylase activity during the late stages of barley grain development and attributed this increase to several factors such as cultivar charateristic, short period of

dormancy, and premature germination.

One way to determine whether or not premature germination has occurred in a cereal grain is to compare the α-amylase isoenzymes isolated from developing and germinated grain. Figure 9 shows such isoenzyme patterns for three stages of development and for germinated grain for the six cereal grains used in the present investigation.

The position on the disc-gel, of equivalent α-amylase isoenzymes, showed some variation for different stages of grain development and among cereal species. This slight variability in the position was attributed to differences in sample composition and to difficulties of the separation and detection techniques. The gels used to examine the extracts of immature grain (12- and 26-day) varied substantially in thickness and consistency after electrofocusing. All immature samples contained some substances (not identified) that softened the gel during electrofocusing. Incorporation of sucrose (10%) into the gel helped to alleviate this problem to some extent. When the β-limit dextrin-acrylamide film (on a glass plate) was placed on top of the disc-gel, containing the separated α-amylases, the weight of the glass compressed the gel to some extent causing some deviations in the position of equivalent isoenzymes. Because of these difficulties identification of the same isoenzymes on different gels is not very reliable. In spite of this operational difficulty, the isoenzymes can certainly be classified into two distinct groups according to their IpH's.

The range in IpH for the separated α-amylases was estimated from an electrofocused blank (sample extract was replaced by distilled water)

Figure 9. Changes in α-amylase isoenzymes during kernel development and germination.

a. 12 days after anthesis.

b. 26 days after anthesis.

c. mature.

d. germinated.

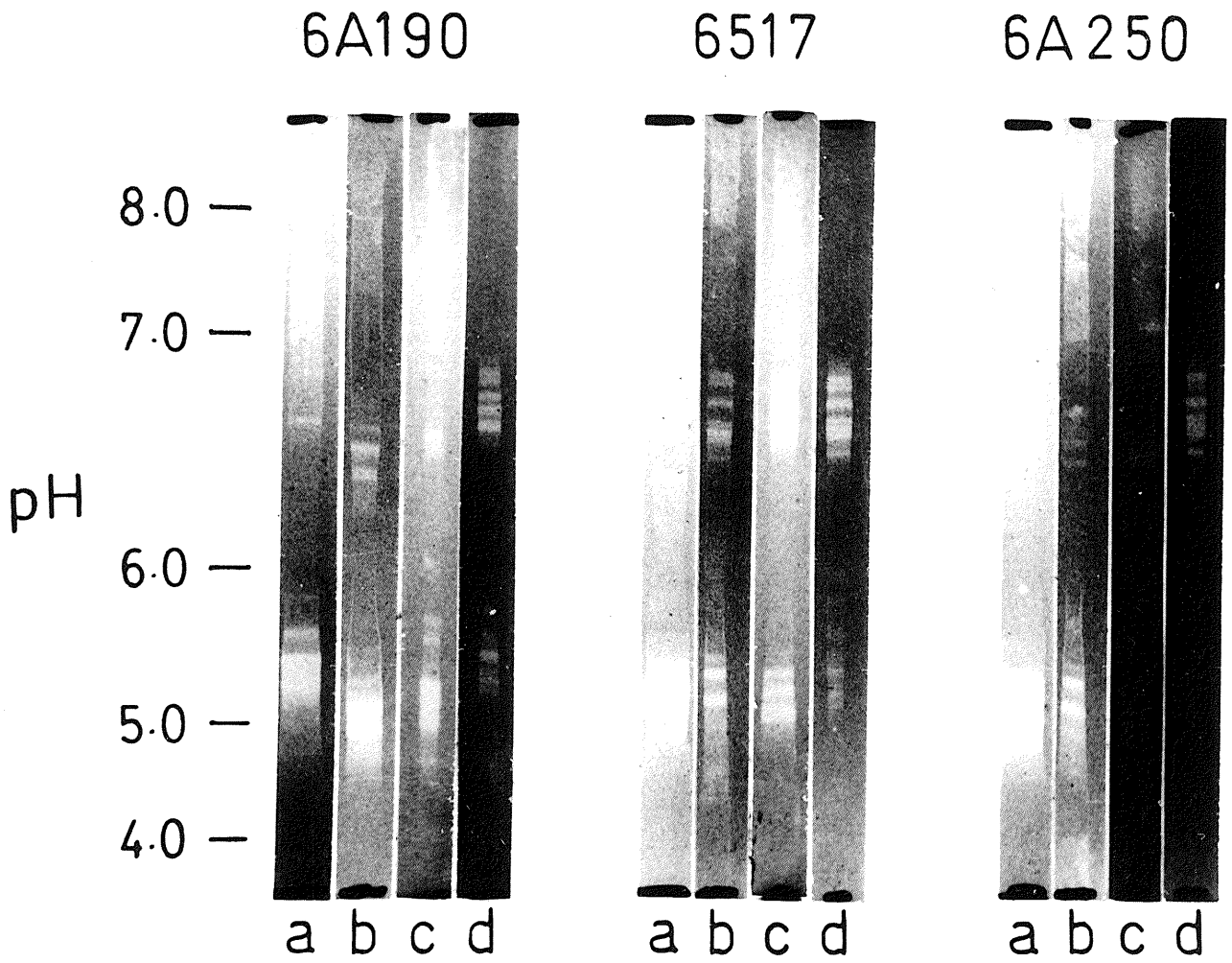
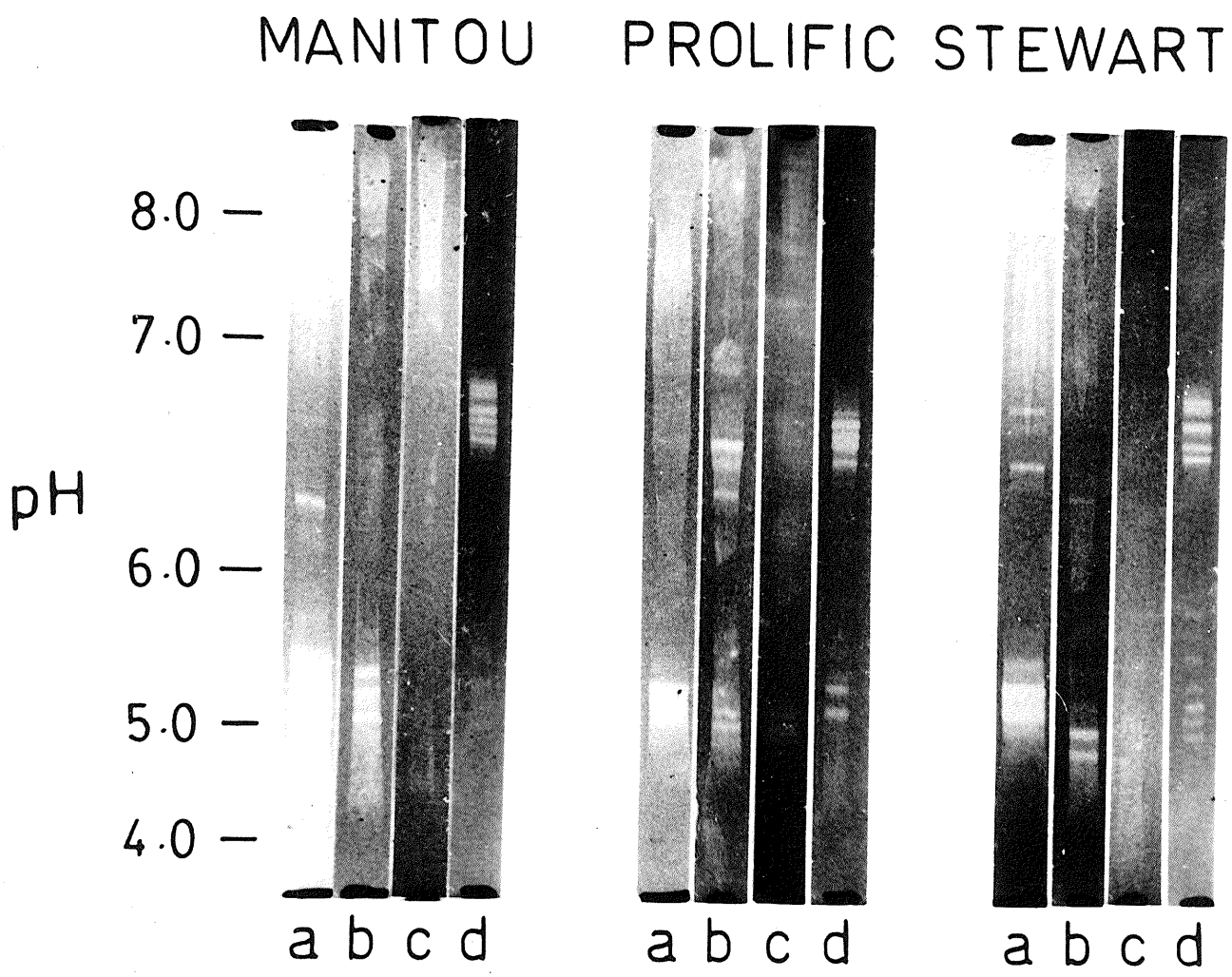

6A190
6517
6A250
8.0
7.0
6.0
5.0
4.0
pH
a b c d
a b c d
a b c d

Figure 9. Continued.


MANITOU
PROLIFIC
STEWART 63
pH
8.0
7.0
6.0
5.0
4.0
a b c d
a b c d
a b c d

disc-gel calibrated in terms of pH. The gel was calibrated by cutting it into 1 cm sections, soaking each section in 2.0 ml of distilled water for approximately 24 hr and then measuring the pH of each solution (resulting from the diffusion of gel components into distilled water). The difference in pH for equivalent gel sections from three such blank gels was less than 0.1 pH unit. The plot of pH versus gel length (or R_f) resulted in an almost linear relationship, except for a slight deviation at about pH 6.0. The blank gel, calibrated for pH, was then used to assign the pH values for the isoenzymes as indicated in Fig. 9.

The low IpH α-amylases have isoelectric points in the pH range from approximately 4.8 to 5.8 and probably correspond to the active α-amylases observed in immature wheat (Kruger, 1972A). The high IpH group (pH from 6.3 to 6.9) appear to be the slow-moving group of α-amylases present in germinated wheat, but absent in mature wheat (Kruger, 1972B). The IpH ranges of the two groups of isoenzymes, noted in the present study, are in general agreement with the findings of Nishikawa and Nobuhara (1971), who also used disc-isoelectric-focusing technique to separate the α-amylase isoenzymes from germinated wheat.

The α-amylase isoenzyme patterns (Fig. 9) varied with grain development and germination. In the HRS wheat for example, the low IpH group of α-amylases showed a progressive decrease in activity (band intensity) with maturation. In the 12-day sample one active high IpH isoenzyme was detected. However, by 26-day, this particular α-amylase was no longer visible, but several new high IpH isoenzymes (with fairly

low activity) appeared. α-Amylase activity could not be detected in mature wheat using the detection procedure outlined in the Methods section. The patterns of α-amylase isoenzymes and changes in activity with maturation, of developing wheat grain, are in general agreement with the patterns obtained by Kruger (1972A), except that no high IpH (electrophoretically slow moving) α-amylases were detected in the earlier study.

For a fair comparison of the α-amylase isoenzyme patterns, of developing and germinated wheat grain (Fig. 9), it must be recalled that gels, containing the electrofocused α-amylases were incubated for 5 and 15 min, respectively for germinated and developing samples. Upon germination the low IpH α-amylases were activated (or synthesized) and a distinct α-amylase pattern developed in the high IpH region that is characteristic of germinated grain. Some of the high IpH isoenzymes appear to be newly synthesized (or activated) while other correspond to isoenzymes present in 26-day sample. If the isoenzyme band intensity reflects α-amylase activity quantitatively, then there is no doubt that in germinated wheat grain the high IpH α-amylases are responsible for the major proportion of the total activity, whereas the low IpH α-amylases contributed more to the activity of developing grain.

There is considerable similarity among the α-amylase isoenzyme patterns for the six cereal grains at each stage of development and for germinated grain. The low IpH α-amylases showed a general decrease in activity with maturation. The triticales and the wheats had one prominent high IpH isoenzyme at 12 days after anthesis. For all cereals examined, the high IpH isoenzymes reached maximum activity at

26 days after anthesis and declined subsequently with maturation. This was particularly clear in the patterns for the rye. Upon germination, many new and active high IpH α-amylases appeared, some have the same IpH's as those of some of the isoenzymes present in immature grain. Triticale 6517 (intermediate shrivelling) had the same isoenzyme patterns for grain harvested at 26 days after anthesis and for germinated grain.

Distinct differences were observed in the isoenzyme patterns of the two triticale lines that yield shrivelled grain and the one that gives plump grain (Fig. 9). In the shrivelled lines, both the high and low IpH α-amylases have similar activities in 26-day and mature grain. In the plump-grained triticale (6A250), the activity of both groups of isoenzymes was much lower in mature grain than in the 26-day grain. In addition, the activities of the high IpH isoenzymes (in 26-day grain) of shrivelled triticales were considerably higher than for equivalent grain of plump triticale.

The results of this section suggest that the large increase in α-amylase activity, during the late stages of grain development, of triticale lines that yield shrivelled grain (Fig. 8), may not be due to premature germination but rather due to lack of suppressing control (e.g. presence of hormones such as gibberellic acid) or presence of metabolite activator(s) of α-amylases that are present in immature grain. The controlling mechanism may be through the action of hormones, such as gibberellic acid. In line with this suggestion Jacobsen *et al.* (1970) showed that gibberellic acid induced the synthesis of specific α-amylase isoenzymes in barley aleurone layers.

Metabolite activators may be compounds such as specific amino acids (glutamate and aspartate) or amino acid intermediates (Galsky and Lippincott, 1971) which are present in the metabolic pool. Obviously further work is needed to determine the actual mechanism of the drastic change in α-amylase activity in shrivelled triticale lines during the final stages of kernel maturation.

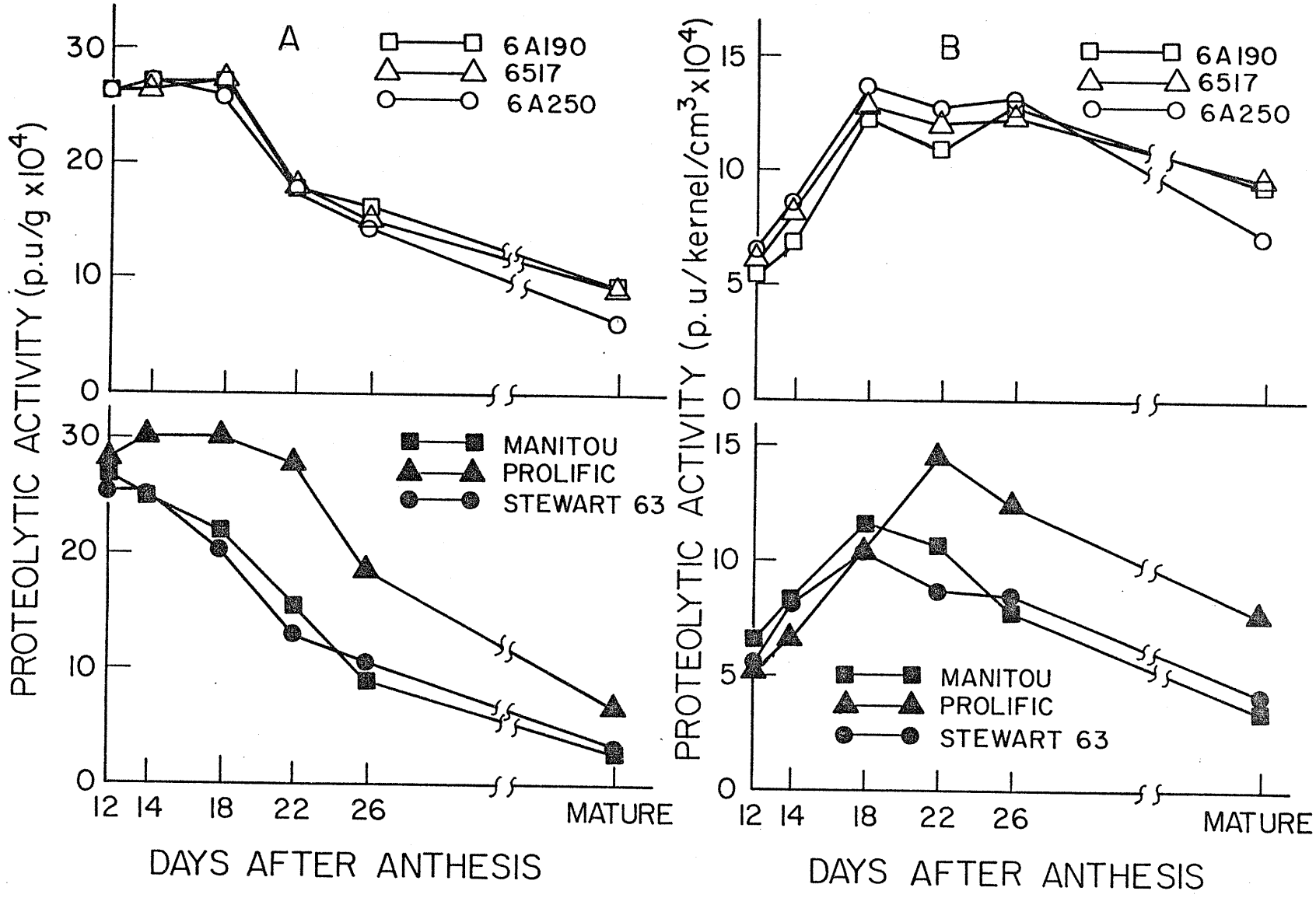
3. Proteolytic Activity

Proteolytic activity of grain at different stages of development for the six cereals are shown graphically in Fig. 10. On a weight basis proteolytic activity of the triticales and rye was constant in the 12- to 18-day (22-day for rye) sample; decreased rapidly from 18 to 22 days (26 days for rye) then more slowly to maturity. The activity of the two wheats decreased rapidly from 12 to 26 days and more slowly thereafter to maturity. In absolute activity, the rye and the triticale samples were about the same (rye was somewhat higher for early stages of development) but higher than the wheat samples.

For the three triticales, there were essentially no differences in proteolytic activity (as expressed in Fig. 10A) that might be related to the degree of shrivelling except at maturity, the two shrivelled triticales 6A190 and 6517 had somewhat higher activity than the plump triticale 6A250.

The trends in proteolytic activity during kernel development in these six cereals examined in this study are in general agreement with

Figure 10. Variations in proteolytic activity during kernel development.


A
B
6A190
6517
6A250
MANITOU
PROLIFIC
STEWART 63
PROTEOLYTIC ACTIVITY (p.u/g x10⁴)
PROTEOLYTIC ACTIVITY (p.u/kernel/cm³x10⁴)
30
20
10
0
15
5
12 14 18 22 26 MATURE
DAYS AFTER ANTHESIS

the findings of Bushuk *et al.* (1971) for developing wheat grain.

Somewhat different shaped curves were obtained when the proteolytic activity was expressed on a per kernel per cm^3 basis (Fig. 10B). All cereals showed an increase in activity during early kernel growth; reaching a maximum level at 18 to 26 days after anthesis. This was followed by a gradual decrease from that stage of development to maturity. The differences in activity among the six cereals are similar to those deduced from the data in Fig. 10A. During the early stages of development (12 to 26 days) the less shrivelled line had the higher activity. A cross-over in the activity between 26-day and maturity did occur so that the two most shrivilled lines had the highest activity at maturity. The curves for the triticales suggest that there may be two peaks of activity, one at 18 days after anthesis and the other at 26 days. During the same developmental period, the rye and the two wheats showed only a single peak in the activity versus maturity curve. The present data on proteolytic activity, expressed on a per kernel per cm^3 basis, yields curves that are similar to those of Kruger (1973) for maturing wheats.

Quantitatively, the triticales resemble rye in proteolytic activity; both species have higher activities than that of wheat at all stages of kernel development. It is concluded from the data presented here that triticale strains that produce grain with relatively different degrees of shrivelling cannot be readily distinguished on the basis of proteolytic activity in the grain.

4. Proteolytic Isoenzymes

The possible implication of the proteases in kernel shrivelling was investigated further by examining the isoenzyme patterns for the grain of increasing maturity for all six cereals used in this study. Representative results are reported for three stages of maturity and for germination (Fig. 11). Four relatively distinct groups of proteolytic isoenzymes were obtained for all cereals. These results are in general agreement with those of Kaminski and Bushuk (1969) and Chua and Bushuk (1969) who found four distinct proteolytic enzymes in sound and malted wheat flours, respectively. The activity of each isoenzyme was highest in the 12-day samples and thereafter progressively decreased with maturation. The germinated samples showed no increase in the intensity of the bands nor any new isoenzymes.

It should be noted that the rather diffuse proteolytic isoenzyme bands limits the sensitivity of the technique. The lack of sharpness of the bands is probably due to the diffusion technique used to incorporate the substrate (hemoglobin) into the gel and to the fairly long digestion period that must be used to detect the relatively low activities.

The isoenzyme patterns of the three triticales are similar; no significant differences were observed in either the activity of the number of isoenzymes (distinct bands). Thus, the slightly higher proteolytic activity of the shrivelled triticales, at maturity, appears to be due to differences in absolute activity and not to the presence

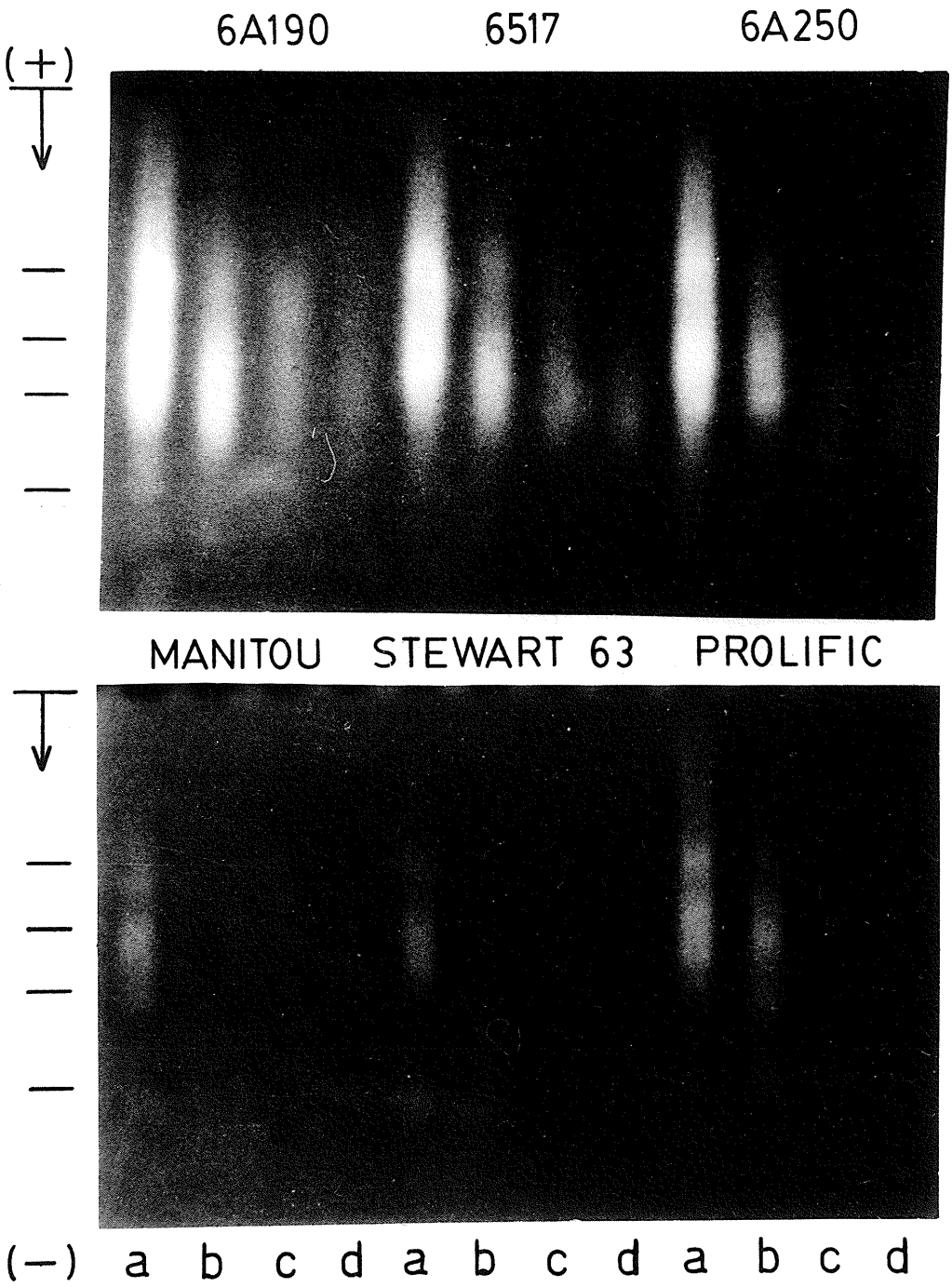
Figure 11. Changes in proteolytic isoenzymes during kernel development and germination.

a. 12 days after anthesis.

b. 26 days after anthesis.

c. mature.

d. germinated.


6A190
6517
6A250
(+)
MANITOU
STEWART 63
PROLIFIC
(−)
a b c d a b c d a b c d

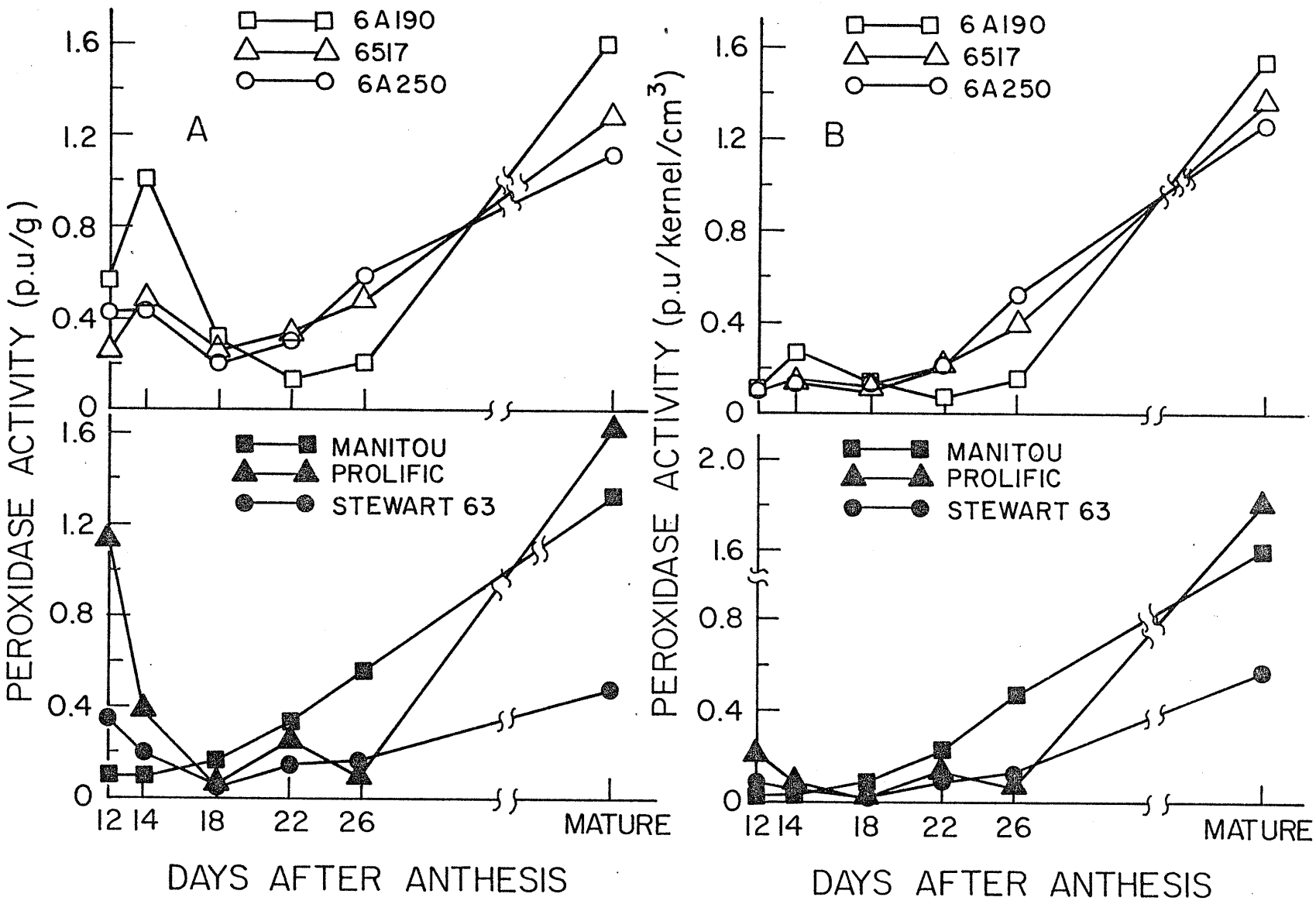
or absence of characteristic isoenzymes. Accordingly, triticales that produce grain of different degrees of shrivelling cannot be distinguished by their protease isoenzyme patterns.

5. Peroxidase Activity

Figure 12A shows the peroxidase activity, on a weight basis, at different stages of kernel maturity. The curves for the six cereals are similar, but some notable differences are apparent. In the shrivelled triticales 6A190 and 6517 peroxidase activity increased between days 12 and 14, then decreased to a minimum between days 18 and 22 and once again increases during the later stages of kernel development. The wheats, rye and triticale 6A250 did not show the initial increase that was readily evident for the shrivelled triticales. Instead, these cereals showed a decrease in activity to a minimum during early development (12 to 26 days). This was followed by an increase in activity with further maturation. Rye had the highest activity both at the early stage of development and maturity. At maturity, the durum wheat had the lowest activity while the triticales and the HRS wheat were intermediate between the rye and the durum wheat.

Among the three triticales there were quantitative differences in activity at the various stages of kernel development. At early maturity peroxidase activity paralleled the degree of shrivelling. This relationship was maintained at 14 and 18 days after anthesis stage, but somewhat reversed at 22 and 26 days after anthesis when the most highly shrivelled triticale (6A190) had the lowest activity.

Figure 12. Variations in peroxidase activity during kernel development.


6A190
6517
6A250
A
B
MANITOU
PROLIFIC
STEWART 63
PEROXIDASE ACTIVITY (p.u/g)
PEROXIDASE ACTIVITY (p.u/kernel/cm3)
1.6
1.2
0.8
0.4
0
2.0
12 14 18 22 26 MATURE
DAYS AFTER ANTHESIS

Another reversal in peroxidase activity occurred between day 26 and maturity. Peroxidase activity of mature grain again paralleled the degree of shrivelling.

Peroxidase activity expressed on a per kernel per cm^3 basis (Fig. 12B) gave similar curves, except that the large variations observed during the early developmental stages were considerably reduced. Again, the six cereals can be clearly distinguished on the basis of their peroxidase activity, particularly at maturity. For the triticales, the activity increased with degree of shrivelling. Of the other three cereals, rye grain was highest in activity, HRS grain was intermediate, and durum wheat was lowest.

The peroxidase activity patterns obtained in the present investigation differ from those obtained by Kruger and LaBerge (1974) for maturing wheats. In this earlier study, peroxidase activity increased to maximum level between days 25 and 30 then decreased with further ripening to maturity. In another earlier study of peroxidase activity in maturing wheats, Bach *et al.* (1926) observed a general increase in activity during early grain growth. Subsequently, the activity levelled-off and remained essentially constant to maturity. Further work is necessary to clarify the reason for the discrepancies among the three studies.

The present study showed that triticale strains that yield grain with different degrees of shrivelling differ quite substantially in the peroxidase activity of mature grain. Because of this relationship, and because of possible implications of peroxidases in grain ripening (Kruger and LaBerge, 1974) this enzyme warrants further investigation

in relation to grain shrivelling in cereals.

6. Peroxidase Isoenzymes

Figure 13 shows the patterns of the anionic (top-A) and cationic (bottom-B) peroxidases for grain at three different stages of kernel development and for germinated grain. A schematic representation of the individual isoenzymes is also included in the figure to show bands that are too faint to be seen on the photograph of the stained gel.

A total of nine anionic peroxidase isoenzymes can be detected for all the cereals (Fig. 13A). These isoenzymes appear to be similar in all cereals, but differences in the intensity of some of the bands are apparent among the cereals. The anionic peroxidases are quite sensitive to repeated freezing and thawing, since the band intensities for frozen samples were considerably lower than those of fresh extracts. Furthermore, these peroxidases showed a general decline in activity with maturation for all cereals studied. The most striking difference among the three triticales was that the fastest moving isoenzyme (1) had the highest activity in the most shrivelled grain.

Upon germination some of the anionic isoenzyme bands increased in intensity (activity). This was particularly evident for bands 1, 2, 3, 8 and 9 in triticales 6517 and 6A250. For the most shrivelled triticale 6A190, only the intensity of band 9 increased with germination.

The anionic isoenzyme pattern of triticale 6A190 for mature and germinated grain resembled the rye patterns more than those of either the other two triticales or the two wheats. The changes in the anionic

Figure 13. Changes in anionic (A) and cationic (B) peroxidase isoenzymes during kernel development and germination.

a. 12 days after anthesis.

b. 26 days after anthesis.

c. mature.

d. germinated.

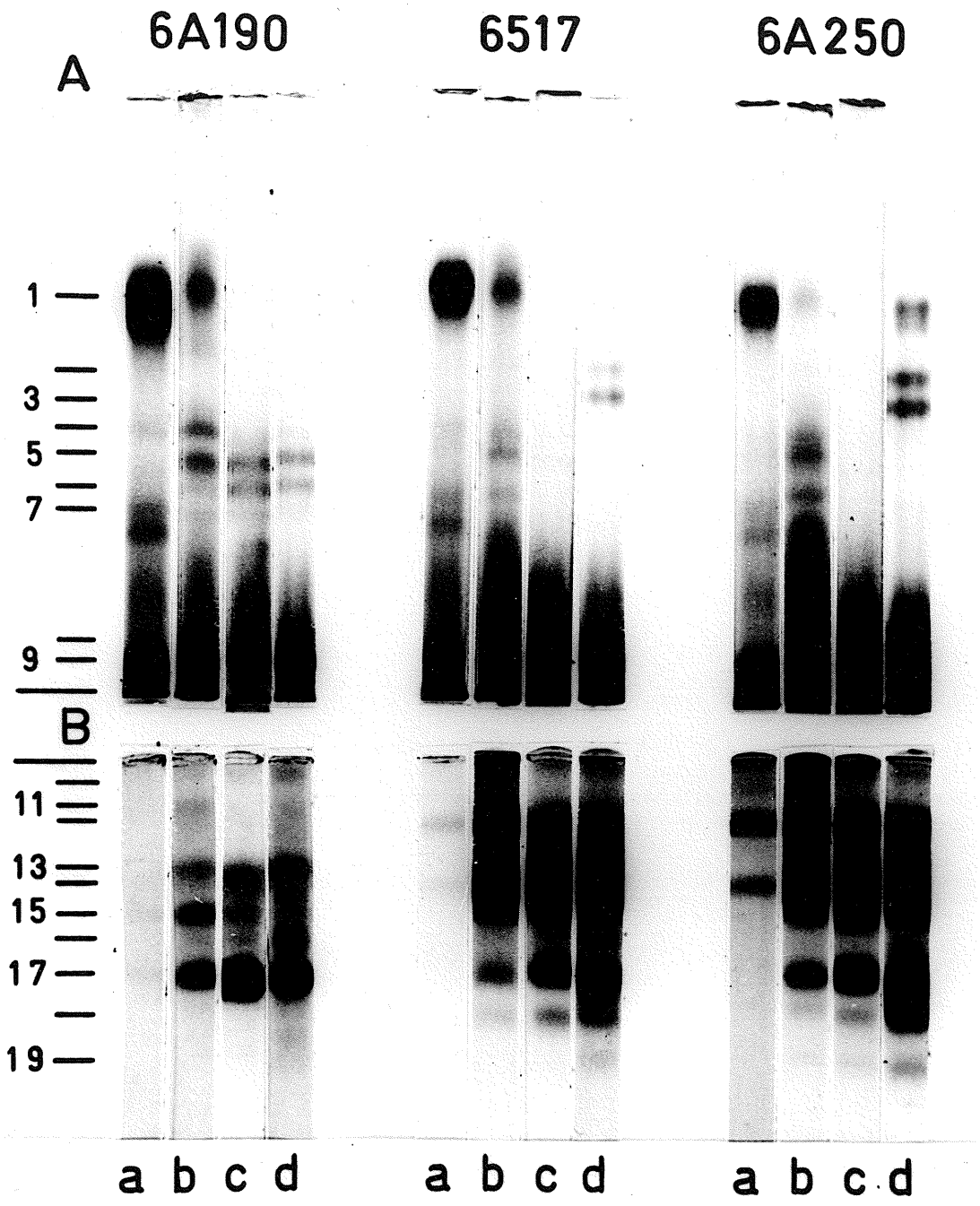
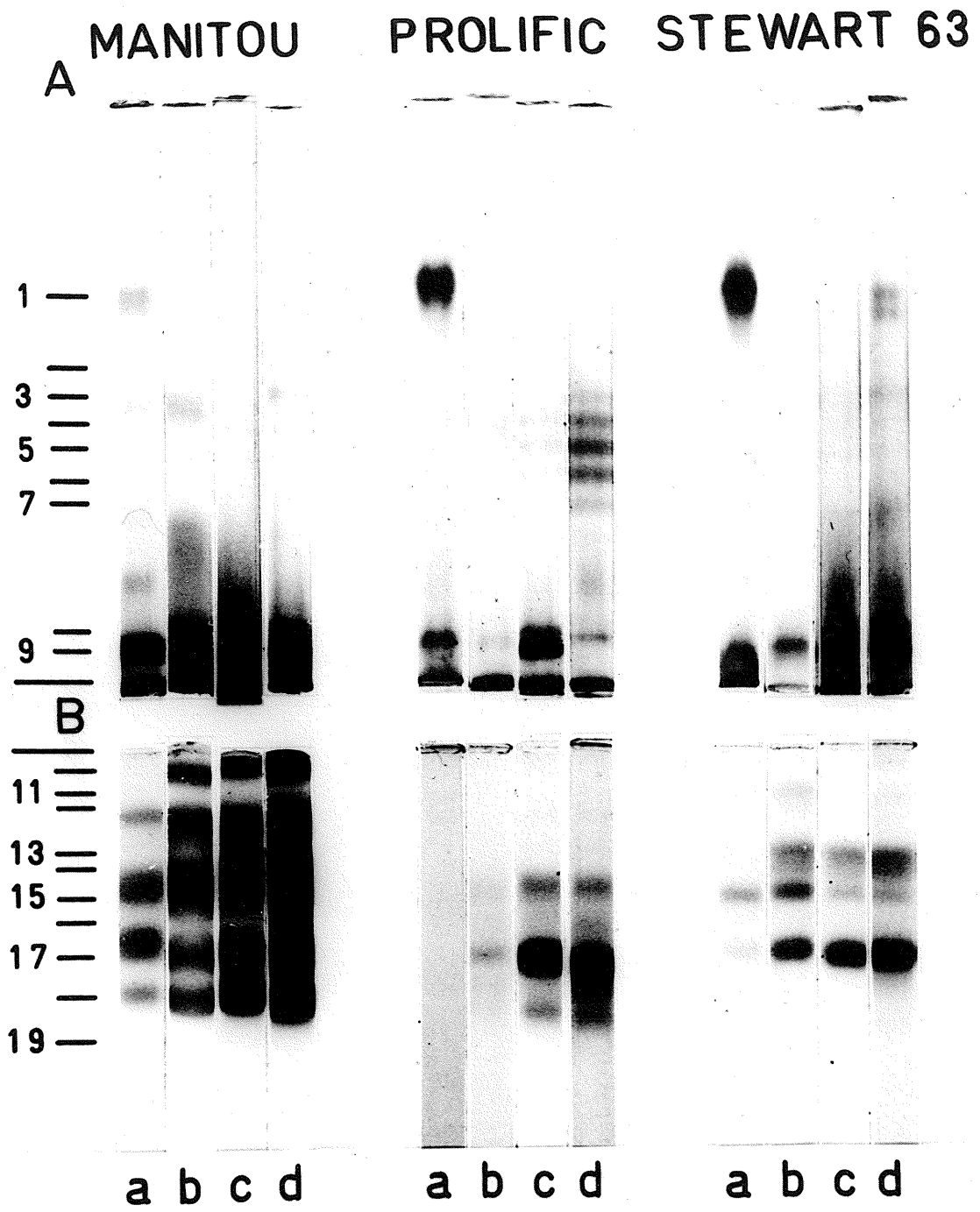

6A190
6517
6A250
A
B
1
3
5
7
9
11
13
15
17
19
a b c d
a b c d
a b c d

Figure 13. Continued.


MANITOU
PROLIFIC
STEWART 63
A
B
1
3
5
7
9
11
13
15
17
19
a b c d
a b c d
a b c d

peroxidases during development and maturation of the two wheats were similar to those in triticales 6517 and 6A250.

The number and the activities (band intensities) of the cationic peroxidase isoenzymes was variable among the six cereals (Fig. 13B). With maturation this group of peroxidases showed a general increase in activity and in the number of isoenzymes for each cereal. Comparison of the HRS wheat and durum wheat patterns confirms the result of Feillet and Kobrehel (1974), who showed that durum wheat lacks the fastest moving cationic isoenzyme (18). Differences between the two wheats were apparent among the slower moving isoenzyme identified by bands 10, 11, 12 and 13.

The low intensity and the number of cationic isoenzymes in triticale 6A190 appears to be inherited from both of its parental species. No "new" or "hybrid" isoenzymes were detected.

In relation to kernel shrivelling the pattern of 6A190 was quite different from the patterns of the other two triticales. The low mobility isoenzymes (11-15) were considerably lower in activity for triticale 6A190 than for the other two strains. The patterns of the three triticales showed the absence (the isoenzyme might be present but of very low activity) of the cationic isoenzyme with highest mobility (19) in the two shrivelled triticales for all stages of kernel development (Fig. 13B). This isoenzyme was present in all three triticales after germination. Further studies are required to firmly establish that isoenzyme 19 is present only in plump triticales. If indeed this observation is generally true, then the presence or absence of this isoenzyme might be a useful sensitive method for screening breeding lines of

triticale for kernel shrivelling.

A general trend for all six cereals is that the anionic peroxidases progressively decreased in activity with maturation, whereas the cationic ones showed a gradual increase. The isoenzyme patterns of germinated grain were similar to the patterns of mature grain.

There is a general lack of information on the specific function of peroxidases in plant metabolism. But, because of their wide occurrence in plants, a number of different functions have been assigned to them. The dramatic changes in isoenzyme activity and composition with maturation suggests that they are extremely important in the development and maturation of cereals grains and thereby may be directly related to (or involved in) kernel shrivelling. This speculation warrants further investigation.

The diffuse regions between anionic peroxidase isoenzyme 7 and 8 (Fig. 13) are due to catalase activity. Catalase can decompose hydrogen peroxide into water and oxygen without the need of a hydrogen donor to accept the oxygen. The catalase isoenzymes were separated by electrophoresis (David, 1964) of a 50 µl aliquot of peroxidase extract and detected by staining the disc gel for catalase according to Woodbury *et al.* (1971). The catalases appeared as yellow bands on a green gel, but were only stable for 15-30 min in light. Figure 14 shows the catalase isoenzyme patterns for mature grain only of all six cereals developed in separate gels. Two catalase isoenzymes of low mobility were observed in each cereal at maturity (and also upon germination, results not shown). These results are analogous to those of Honold and Stahmann (1968) who found two closely migrating catalases

Figure 14. Catalase isoenzymes of mature grains.

a. triticale 6A250.

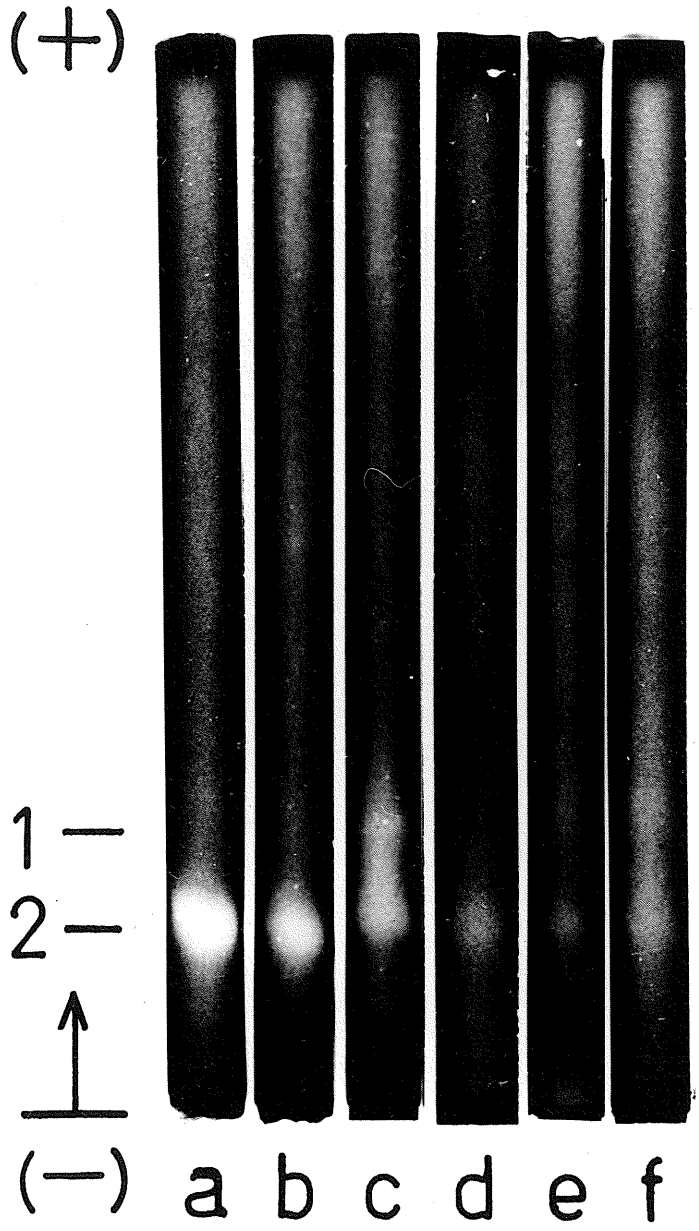
b. triticale 6517.

c. triticale 6A190.

d. Prolific spring rye.

e. Stewart 63 durum wheat.

f. Manitou HRS wheat.


(+)
1
2
(−)
a b c d e f

in four wheat varieties. The fast moving isoenzyme (band 1) could not be detected during early development in any of the cereals and even at maturity it was present at very low activity in most of the six cereals. In the pattern for triticale 6A190 both isoenzymes are recognizable in Fig. 14 for mature grain. The intensity (activity) of the slow moving isoenzyme (band 2) was low during early kernel development but increased with maturity and was readily detectable in all six cereals. Upon germination, the activity of this slow moving isoenzyme increased considerably. The activity of this slow moving catalase in mature grain decreased slightly as the degree of grain shrivelling in triticale increased (see Fig. 14).

D. Proteins

1. Gel Filtration Chromatography

The proteins from grain harvested at different stages of development were examined for possible differences in molecular weight distribution, as determined by gel filtration. The proteins were extracted with AUC solvent and the extracts subjected to gel filtration chromatography of Sephadex G-150 using the extracting solvent as the eluant. This solvent solubilizes approximately 95% of the wheat endosperm protein and a somewhat higher proportion of the more soluble rye and triticale endosperm protein.

Five distinct elution peaks were obtained for all cereals at the various stages of development (Fig. 15). For 12-day samples, all six cereals showed similar elution profiles. Differences in the elution profiles were apparent for 26-day samples. At this stage of

Figure 15. Gel filtration elution curves of AUC extracts of grain at various stages of development and of flour. Figures on top indicate elution position of proteins of given MW and position of the Roman numerals represent the five distinct elution peaks.

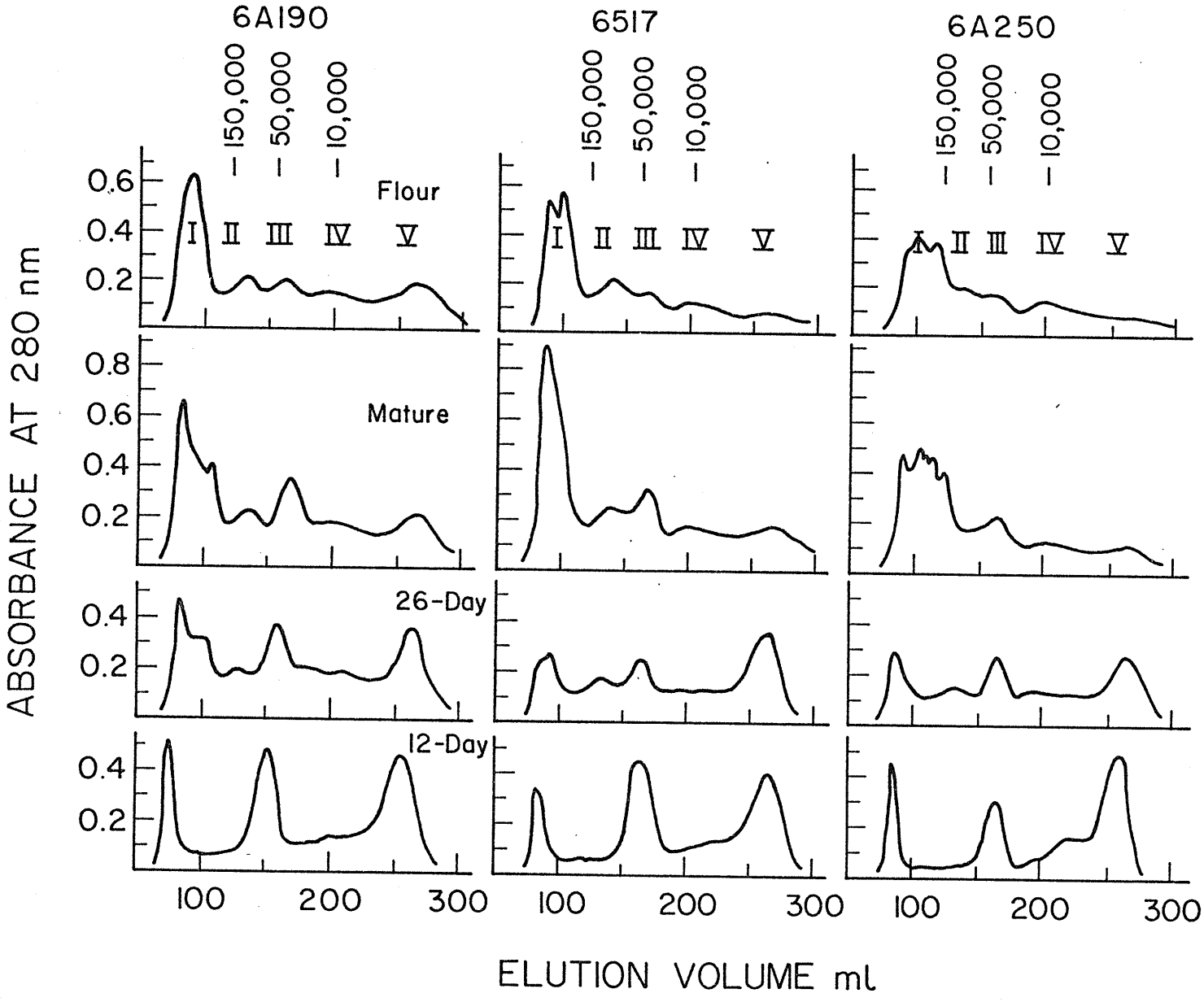
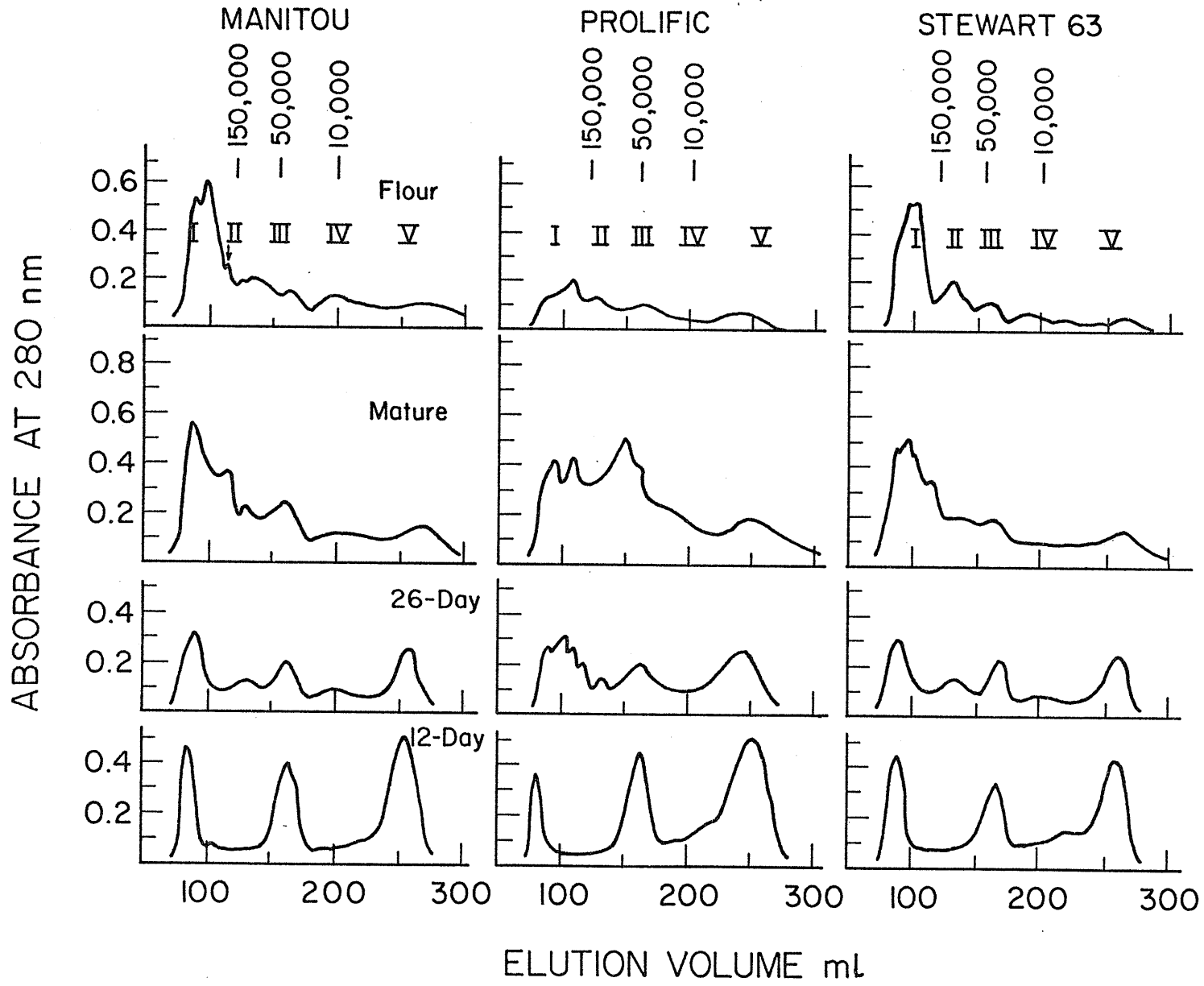

6A190
6517
6A250
150,000
50,000
10,000
Flour
Mature
26-Day
12-Day
I
II
III
IV
V
0.6
0.4
0.2
0.8
100
200
300
ABSORBANCE AT 280 nm
ELUTION VOLUME ml

Figure 15. Continued.


MANITOU
PROLIFIC
STEWART 63
150,000
50,000
10,000
I
II
III
IV
V
Flour
Mature
26-Day
12-Day
0.6
0.4
0.2
0.8
100
200
300
ABSORBANCE AT 280 nm
ELUTION VOLUME ml

development, the peak(s) for the high molecular weight (MW) fraction showed evidence of the characteristic shape of the profiles of mature grain. For mature grain, major differences in the profiles of the six cereals is in the high MW region (peaks I and II).

The elution profiles for the gel filtration of extracts of flour milled from mature grain of each species are included in Fig. 15 for comparison with the profiles of extracts of whole meal. Minor qualitative and quantitative differences between the profiles of the flour and meal extracts were observed for some of the cereals. These were not investigated further.

The elution profiles for the two wheats were quite similar for all samples (different developmental stages and flour) that were examined. This differs somewhat from the results of Bushuk and Wrigley (1971) whose elution profiles for HRS and durum wheats differed distinctly in the high MW region. However, the peak or shoulder in the elution curve corresponding to a MW of approximately 230,000 (marked by arrow) is present in the elution profile of the extract of HRS flour but absent in the durum flour extract as found by Bushuk and Wrigley (1971).

The gel filtration curves for the three triticales showed deviations that suggest qualitative and quantitative differences in the proteins of extracts of grain harvested on a specific date. The differences were quite significant for mature grain (Table 6). When the elution peak areas were expressed as a percentage of the total under the elution curve, the area of peak I and II (high MW proteins) showed a decreasing trend with increase in the degree of kernel shrivelling.

TABLE 6. PEAK AREA PERCENTAGE OF TOTAL ELUTION CURVE OF MATURE WHOLE MEAL.

Cereal species	% of total peak area*		
	(I + II)	III	(IV + V)
Triticale 6A190	48.9	17.1	34.0
Triticale 6517	52.5	13.1	34.4
Triticale 6A250	57.3	15.0	27.7
HRS wheat	53.8	16.0	30.2
Durum wheat	57.3	11.6	30.5
Spring rye	28.8	27.7	37.5

* Result of a single determination.

The area of peak III showed little variation among the triticales. The area of peaks IV and V (the low MW proteins) were considerably higher for the shrivelled triticale lines 6A190 and 6517. For grain harvested at early stages of development (12 and 26 days) there was no trend between any of the peak areas and kernel shrivelling.

The proportion of protein in the high MW fraction of the AUC extractable proteins is directly related to breadmaking quality of wheat (Orth and Bushuk, 1973). Accordingly, the observed higher proportion of peak I and II proteins in the less shrivelled triticale lines may be relevant to the utilization of triticale flour for bread production. This point warrants further investigation.

Among the six cereals that were studied, the high MW fraction (peaks I and II) increased gradually with maturation, while the low MW fraction (peaks IV and V) decreased. The intermediate fraction (peak III) showed a slight decreasing trend with maturation. The increase in the high MW fraction parallels the well-known development of breadmaking properties of wheat with kernel maturation.

Comparison of the quantitative distribution of various MW species for triticale grain harvested at 12 days post anthesis and maturity (Table 7) shows that a drastic change occurs in the MW of the endosperm proteins during development and maturation. The differences observed may be related to differences in metabolism in lines that yield shrivelled and plump kernels. For example, the large decrease in peak V for triticale 6A250 (the plump-seeded line) suggests a more efficient utilization of these low MW substrates (for the synthesis of the high MW proteins) during maturation of this line. Peaks III

TABLE 7. DIFFERENCES IN ELUTION CURVE PEAK AREAS FOR 12-DAY AND MATURE SAMPLES.

Cereal species	Elution curve peak area (12-day - mature)			
	(I + II)	III	IV	V
	%	%	%	%
Triticale 6A190	-25.9	- 7.1	2.6	16.3
Triticale 6517	-32.1	-15.8	0.7	15.5
Triticale 6A250	-34.9	- 4.1	5.5	25.4
HRS wheat	-27.4	- 7.5	-2.7	22.5
Durum wheat	-28.2	- 9.2	5.0	14.4
Spring rye	-10.9	1 3.2	-1.1	21.2

and IV include many enzymes. Quantitative differences in these fractions may be of major importance in kernel development. The observed changes in the amounts of the high MW components (peaks I and II) during development indicates a slower development of these components in the more shrivelled triticales.

2. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was used to examine the AUC protein extracts. The extracts of grain at three (12-, 26-day and maturity) stages of kernel development and flour milled from mature grain were used without and with prior reduction of disulfide bonds by β-mercaptoethanol. MW's of the components were estimated from a calibration curve obtained for known proteins under identical conditions.

Figure 16 shows the SDS-PAGE patterns of the non-reduced proteins for all six cereals for three stages of development and for flour. Under these conditions (no reduction) a major proportion of the protein does not enter the gel and remains at the origin. The proteins that do enter the gel under the conditions used range in MW from approximately 10,000 to 400,000 daltons. Since many of the minor bands showed extremely low intensities, a schematic representation of bands is included in Fig. 16.

Comparing the relative band intensities, the largest proportion of the proteins that enter the gel have MW's between 10,000 and 50,000. This was generally true for all six cereal species. The bands for the 12-day grain are quite diffuse, but by day 26 the bands are distinct.

The SDS-PAGE patterns of the non-reduced proteins reveal that

Figure 16. SDS-PAGE patterns of non-reduced AUC protein extracts of grain during development and of flour.

a. 12 days after anthesis.

b. 26 days after anthesis.

c. mature.

d. flour of mature grain.

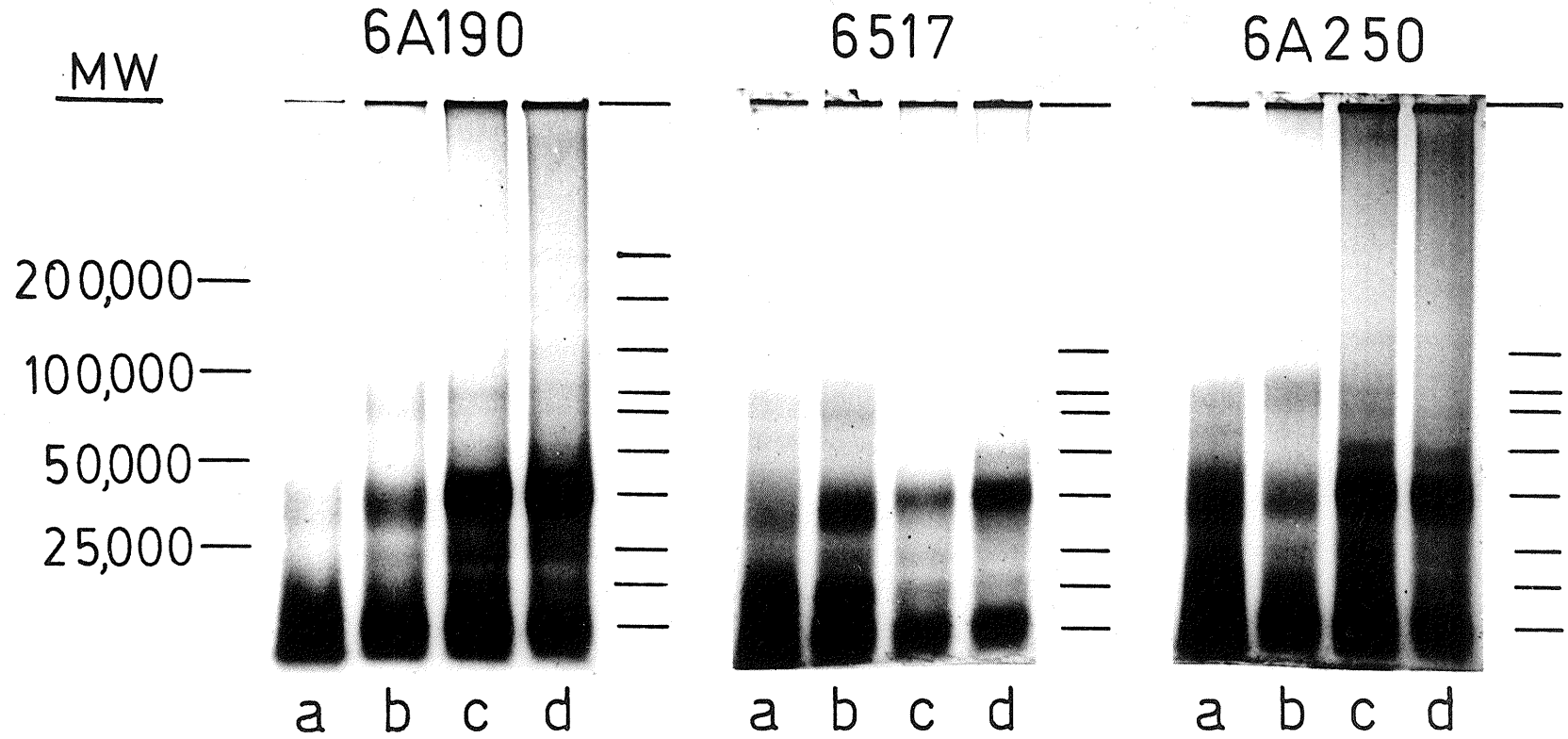
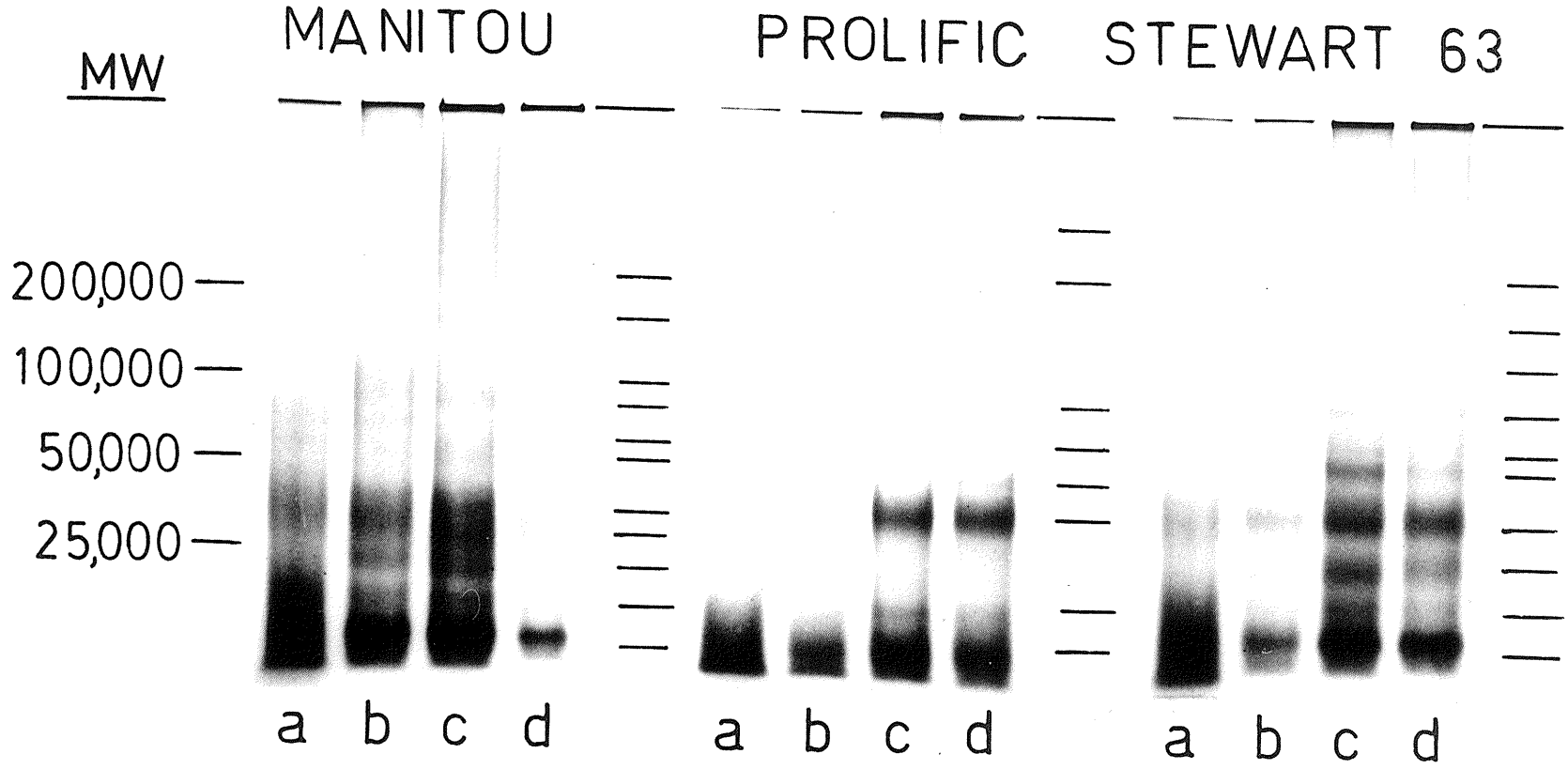

MW
6A190
6517
6A250
200,000
100,000
50,000
25,000
a b c d
a b c d
a b c d

Figure 16. Continued.


MW
MANITOU
PROLIFIC
STEWART 63
200,000
100,000
50,000
25,000
a b c d
a b c d
a b c d

both quantitative and qualitative changes occur in the proteins (that enter the gel) during development. The most significant change is that the number of high MW components increased with maturation for most of the species that were studied. This is particularly evident for the extracts of triticale 6A190 and HRS wheat (Manitou); the bands with MW greater than 200,000 are quite distinct in the photograph. All non-reduced proteins with MW greater than 150,000 are not present in extracts that have been reduced with β-mercaptoethanol (see Fig. 17, later). The SDS-PAGE patterns of the non-reduced AUC extracts of the three triticales are similar for all stages of development and for flour. The line 6A190 appears to have more high MW components than the other triticales. No distinct differences were observed by this technique between plump and shrivelled triticales.

The patterns for flour were included for comparison purposes. The patterns for whole meal and flour of mature grain were essentially the same except for minor differences in the intensity of the stained bands.

Figure 17 shows SDS-PAGE patterns of reduced (β-mercaptoethanol) AUC-soluble proteins for the six cereal species studied. As in Fig. 16, results represent three stages of kernel development and flour milled from mature grain.

Bands that represent proteins with MW's greater than 25,000 increased in intensity with maturation for all species. This trend was particularly apparent for proteins with MW's in the range 75,000 to 150,000. The patterns of this group of high MW proteins (or subunits after reduction) are characteristic of the cereal species and resemble gliadin (Preston and Woodbury, 1976) and/or glutenin (Orth *et al.*, 1974)

Figure 17. SDS-PAGE patterns of reduced AUC protein extracts of grain during development and of flour. Identity of patterns a to d is the same as in Fig. 16.

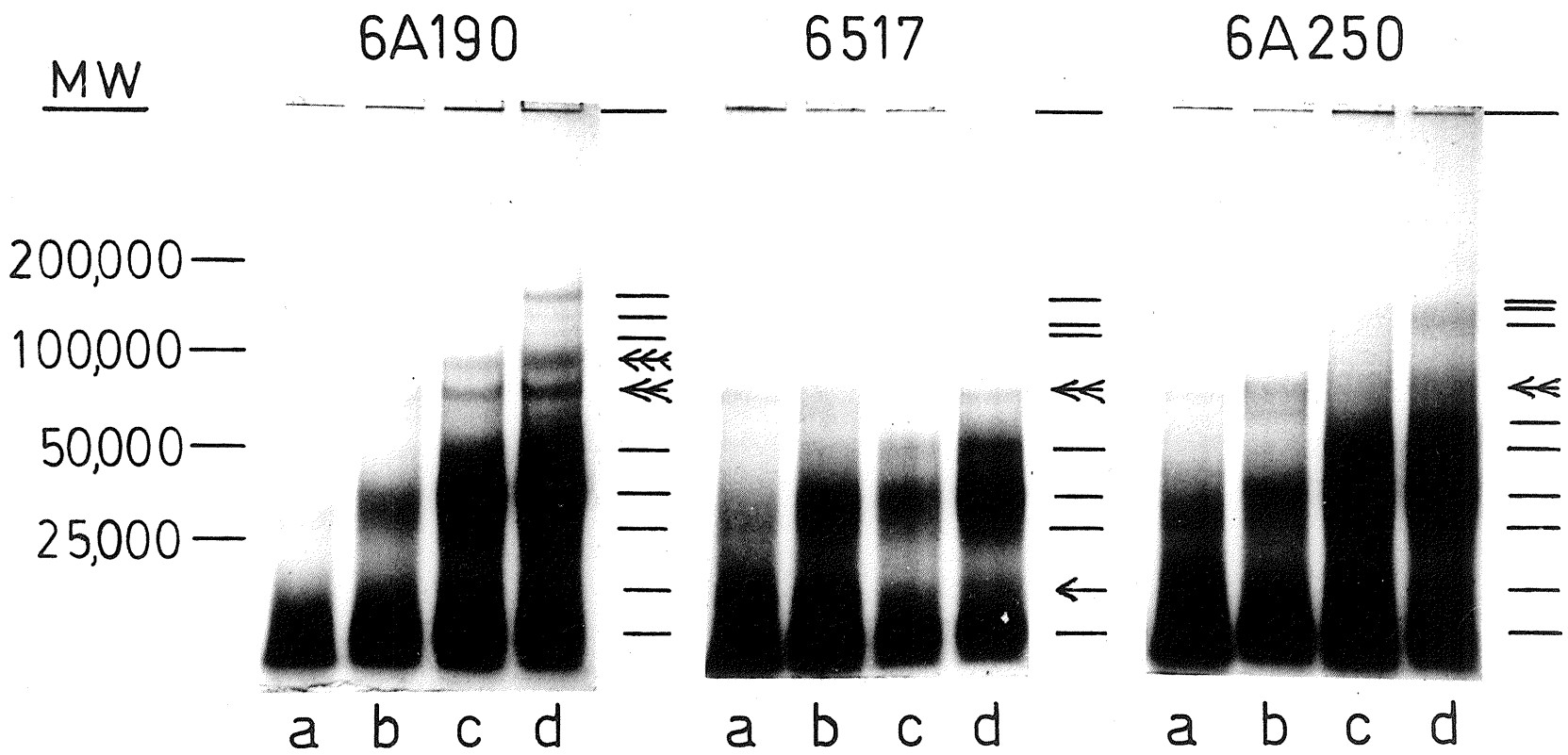
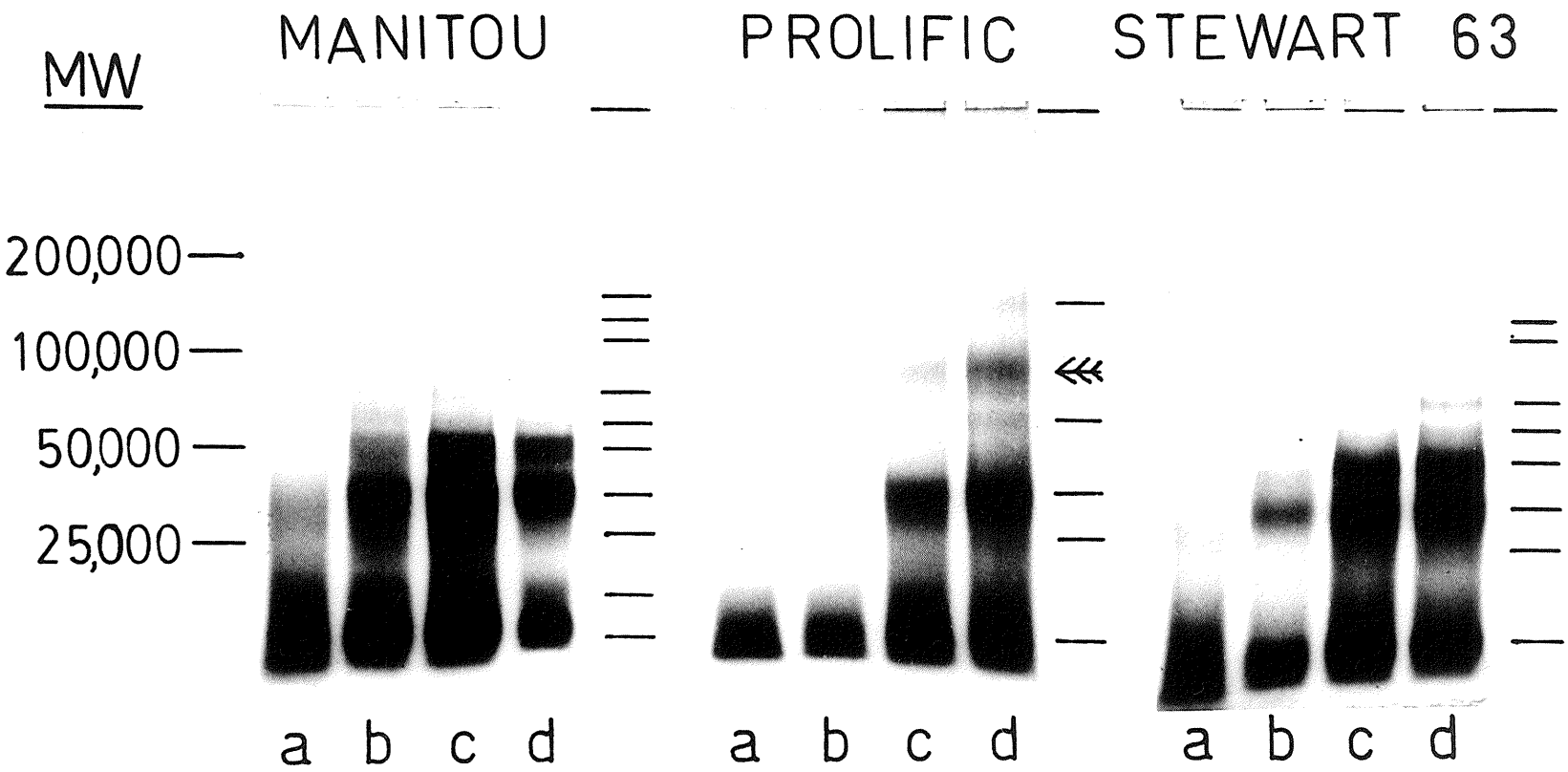

MW
6A190
6517
6A250
200,000
100,000
50,000
25,000
a b c d
a b c d
a b c d

Figure 17. Continued.


MW
MANITOU
PROLIFIC
STEWART 63
200,000
100,000
50,000
25,000
a b c d
a b c d
a b c d

subunits.

The patterns for the three triticales are similar for all stages of kernel development but differences, in intensity and position, do exist among the high MW subunits. In addition, the band representing a low MW subunit (shown by single arrow) in immature triticale 6517 was particularly intense compared with the equivalent subunit of the other triticales. The 72,000 subunit (marked by double arrow) was much more prominent in the patterns for the shrivelled triticales than in the patterns for the plump triticale 6A250. The 79,500 subunit (indicated by triple arrow) was present in the patterns for the rye and triticale 6A190 but is absent in the patterns for the other two triticales. A detailed examination of the results in Fig. 17 showed that the pattern for triticale 6A190 did not show any bands that were not present in the patterns for its parental cultivars, Prolific rye and Stewart 63 durum wheat. This is in general accord with previous findings of Chen and Bushuk (1970C) by disc polyacrylamide gel electrophoresis that the proteins in primary triticales are simply inherited from its parents.

3. Solubility Fractionation

The results of the Osborne solubility fractionation for the six cereals used in the present investigation at three stages of kernel maturity are presented in Fig. 18. Also, included in these figures are the solubility data for the flours milled from mature grains, to determine to what extent the bran and germ components alter the solubility distribution of endosperm protein. The percentage of

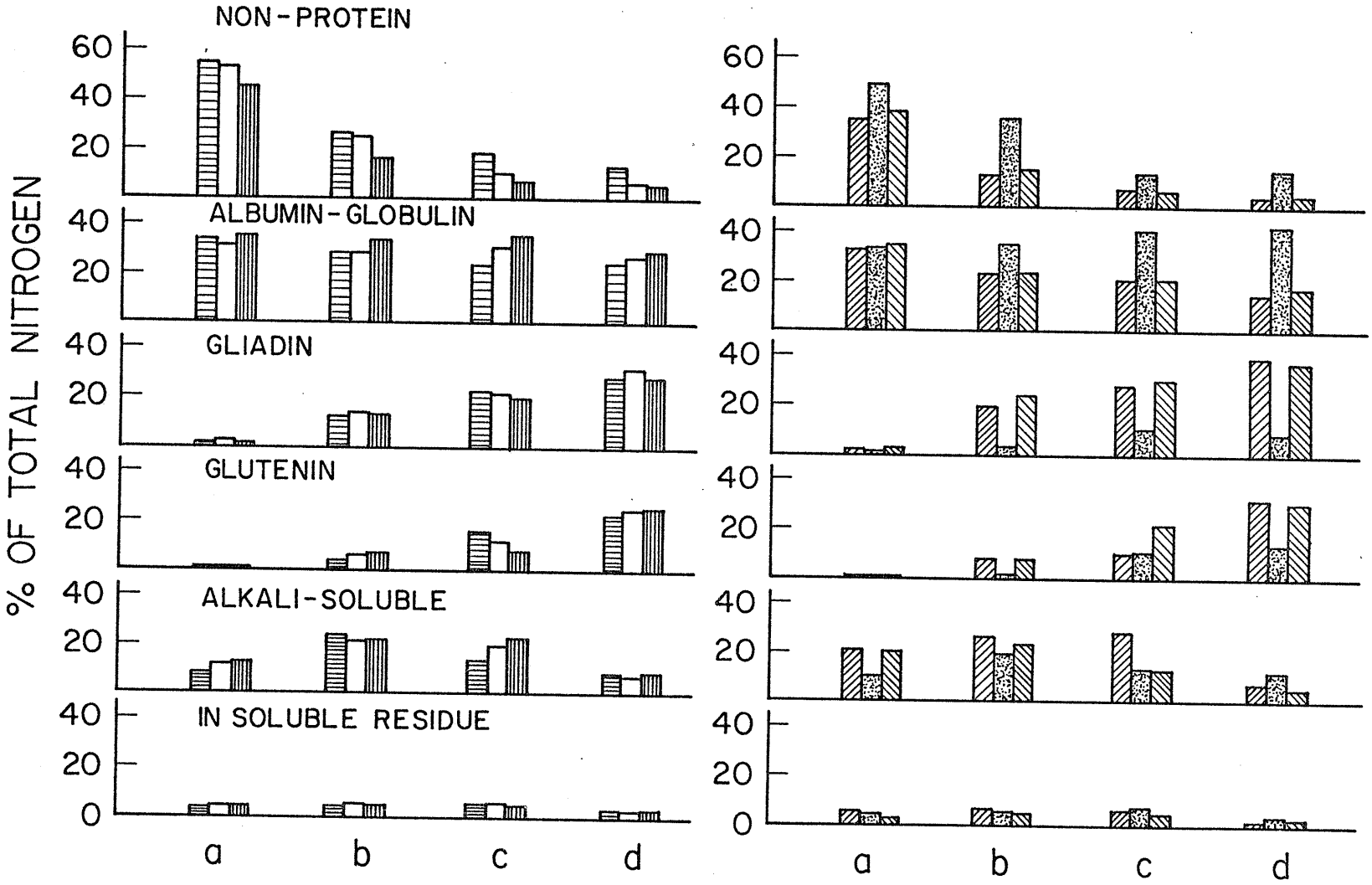
Figure 18. Nitrogen solubility distribution of grains during development and of flour [6A190 (), 6517 (), 6A250 (), Manitou (), Prolific (), and Stewart 63 ()].

a. 12 days after anthesis.

b. 26 days after anthesis.

c. mature.

d. flour of mature grain.


NON-PROTEIN
ALBUMIN-GLOBULIN
GLIADIN
GLUTENIN
ALKALI-SOLUBLE
IN SOLUBLE RESIDUE
% OF TOTAL NITROGEN
60
40
20
0
a
b
c
d

each protein was calculated from its nitrogen as percentage of total nitrogen. This eliminates the need to use nitrogen to protein conversion factors for each fraction, which may not be a constant value of 5.7.

Differences among the solubility distributions were observed within a species for different stages of maturity and among the species at a particular stage of maturity. These results are in general agreement with analogous results for maturing wheat (Feillet, 1965), barley (Ivanko, 1971), triticale and its parental species (Dexter and Dronzek, 1975A).

The proportion of non-protein nitrogen decreased with kernel maturation in all cereals. For a specific stage of kernel development, large differences were obtained in this fraction among the various cereals. The high non-protein nitrogen content of the triticales resembled that of rye. The two wheats had considerably less of this fraction.

The three triticales contained somewhat different proportions of non-protein nitrogen. As the degree of grain shrivelling increased the proportion of this fraction increased for all stages of kernel growth. These results suggest that the metabolism of the non-protein nitrogen components in the plump triticale is more efficient than in the more shrivelled triticales. The difference between the non-protein nitrogen values for mature whole meal and flour indicates that the non-endosperm parts of the kernel contain more of this nitrogen fraction than the endosperm.

The proteins extracted with a 0.5M salt solution were not

separated further; this fraction comprises the albumin and globulin proteins. Rye samples showed a slight increase in the proportion of this fraction with maturation, in contrast to the two wheats which showed a slight decreasing trend. The proportion of this fraction was almost identical in the two wheats throughout maturation. At 12 and 26 days after anthesis and at maturity the proportion of this albumin-globulin fraction was 33.2, 34.7 and 40.8% respectively in rye, whereas in the HRS wheat it represented 33.7, 23.5 and 20.7%, respectively.

The most shrivelled triticale 6A190 showed a slight decrease in the proportion of the salt-soluble fraction with maturation while for the other two triticales it remained relatively constant. The plump triticale 6A250 had the highest percentage of this fraction at all stages of kernel growth. For the three triticales, the proportion of this fraction decreased as the degree of kernel shrivelling increased.

Table 8 gives the relative proportions of the salt-soluble fraction in mature whole meal and flour for all six cereals and the difference between the values. The difference between the meal and flour values appears to be inversely related to kernel shrivelling. The values for the highly shrivelled line (6A190) was similar to that for rye whereas the values for the other two triticales were similar to those for the wheats.

The proportion of the gliadin fraction increased with kernel maturation for all cereals. Large differences in the proportion of gliadin proteins were obtained among the cereals. For example, the

TABLE 8. PERCENTAGE OF THE SALT-SOLUBLE FRACTION IN MATURE WHOLE MEAL AND FLOUR SAMPLES.

Cereal species	Meal	Flour	Meal-Flour
	%	%	%
Triticale 6A190	22.7	24.1	-1.4
Triticale 6517	30.5	26.7	3.8
Triticale 6A250	35.0	28.6	6.4
HRS wheat	20.7	14.9	5.8
Durum wheat	21.0	17.4	3.6
Spring rye	40.8	42.4	-1.6

percentage of this fraction in rye increased from 0.4 to 10.5% between day 12 and maturity, whereas in the HRS wheat, the analogous figures were 1.9 and 28.0%. The percentage of gliadin in the triticales was intermediate between the values for rye and the wheats for all stages of kernel maturity.

The proportions of the gliadin fraction for the triticales were similar at all stages of kernel growth. For mature grain, the percentages were 22.4, 21.6 and 19.5% for 6A190, 6517 and 6A250, respectively. Thus, the proportion of gliadin increased somewhat as the degree of kernel shrivelling increased.

The proportion of gliadin in flour for all cereals except rye was higher than in mature whole meal. This is consistent with the fact that storage proteins are localized primarily in the endosperm component of the grain.

The proportion of the soluble glutenin (acetic acid-soluble fraction) showed an increasing trend with kernel development similar to that obtained for gliadin. At 12 days after anthesis the grain of all species studied did not have any acetic acid-soluble glutenin. This suggests that the high MW proteins (observed by gel filtration) for this early stage of kernel development is of the acetic acid insoluble or residue fraction.

In mature grain the highest proportion of soluble glutenin was found for the durum wheat which contained 22.0%, whereas triticale 6A250 had the lowest soluble glutenin content of 7.7%. The proportion of soluble glutenin in the flours was considerably higher than in the whole meal, indicative again of the fact that this fraction is a

storage protein.

Differences in the proportion of this glutenin fraction were apparent among the various cereals. As for the three triticales, the proportion of soluble glutenin, in 26-day grain, was lowest in the most shrivelled grain. For mature grain, this relationship was reversed.

After the extraction of the soluble glutenin fraction with dilute acetic acid, the residue was further extracted with a 0.1M sodium hydroxide solution. The proportion of this alkali-soluble fraction increased with maturation for HRS wheat and triticale 6A250, whereas for the other four cereals it increased to a maximum level at day 26 and then decreased with further maturation. In flour, the proportion of this fraction was similar for the six species and was considerably lower than in the whole meals from mature grain.

For the three triticales the percentage of the alkali-soluble fraction was lower in the more highly shrivelled grain.

The percentage of the acetic acid and alkali soluble fractions increased with maturation for all six cereals (Table 9). The proportion of the combined fraction did not show any obvious relationship to the degree of kernel shrivelling for the three triticales.

A small percentage of the total nitrogen (1.6 to 6.7%) remained in the residue after extraction with sodium hydroxide. The nature of the constituents containing this nitrogen was not investigated.

The data presented in Figure 8 and Tables 8 and 9 are the average of duplicate extractions. The difference between duplicate extractions ranged from 0 to 2.9 percentage units with an average

TABLE 9. COMBINED PERCENTAGE OF THE ACETIC ACID AND ALKALI SOLUBLE FRACTIONS DURING KERNEL DEVELOPMENT.

Cereal Species	% of total nitrogen		
	A	B	C
Triticale 6A190	8.3	27.3	29.3
Triticale 6517	11.5	27.1	29.9
Triticale 6A250	12.7	28.4	29.8
HRS wheat	21.3	34.0	37.5
Durum wheat	20.3	31.2	35.4
Spring rye	10.3	20.7	24.0

A. 12 days after anthesis.

B. 26 days after anthesis.

C. Mature.

experimental error of 0.4 percentage units. Nitrogen recovery by the fractionation procedure varied from 94.7 to 100.3% with a mean value of 97.9%. The high recovery (97.9%) and the relatively low experimental error (±0.4 percentage units) are reported in support of the claims made above.

4. Electrophoresis of Protein Fractions Obtained by Solubility Fractionation

The protein fractions obtained by the modified Osborne fractionation procedure were analyzed by polyacrylamide gel electrophoresis. The patterns of the fractions of the 12-day samples did not show any distinct bands for any of the species. Accordingly, these patterns were not included in any of the figures pertinent to this section.

Figure 19 shows the electrophoretic patterns for the four protein fractions (of triticale 6A250) of whole grain at two stages of grain development, 26 days and complete maturity, and of flour milled from mature grain. The change in the electrophoretic patterns with maturation was mainly in the intensity of some specific bands (i.e. in the amount of a particular component). The electrophoretic patterns of the fractions from whole meal and flour were essentially the same for all six cereals. There were no obvious differences in the patterns for the three triticales that can be co-related to kernel shrivelling.

Figure 20 shows the polyacrylamide gel electrophoresis patterns of the albumin-globulin fractions from mature whole meal samples, electrophoresed for 60 (I) and 180 (II) min. The longer running time was used to improve the resolution.

The number of distinct components varied significantly among the

Figure 19. Electrophoretic patterns of the salt-(A), alcohol-(B), acetic acid-(C) and alkali-soluble (D) fractions for 26-day (a), mature (b) and flour (c) samples of plump triticale 6A250. (Electrophoresis was carried out for 60 min.)

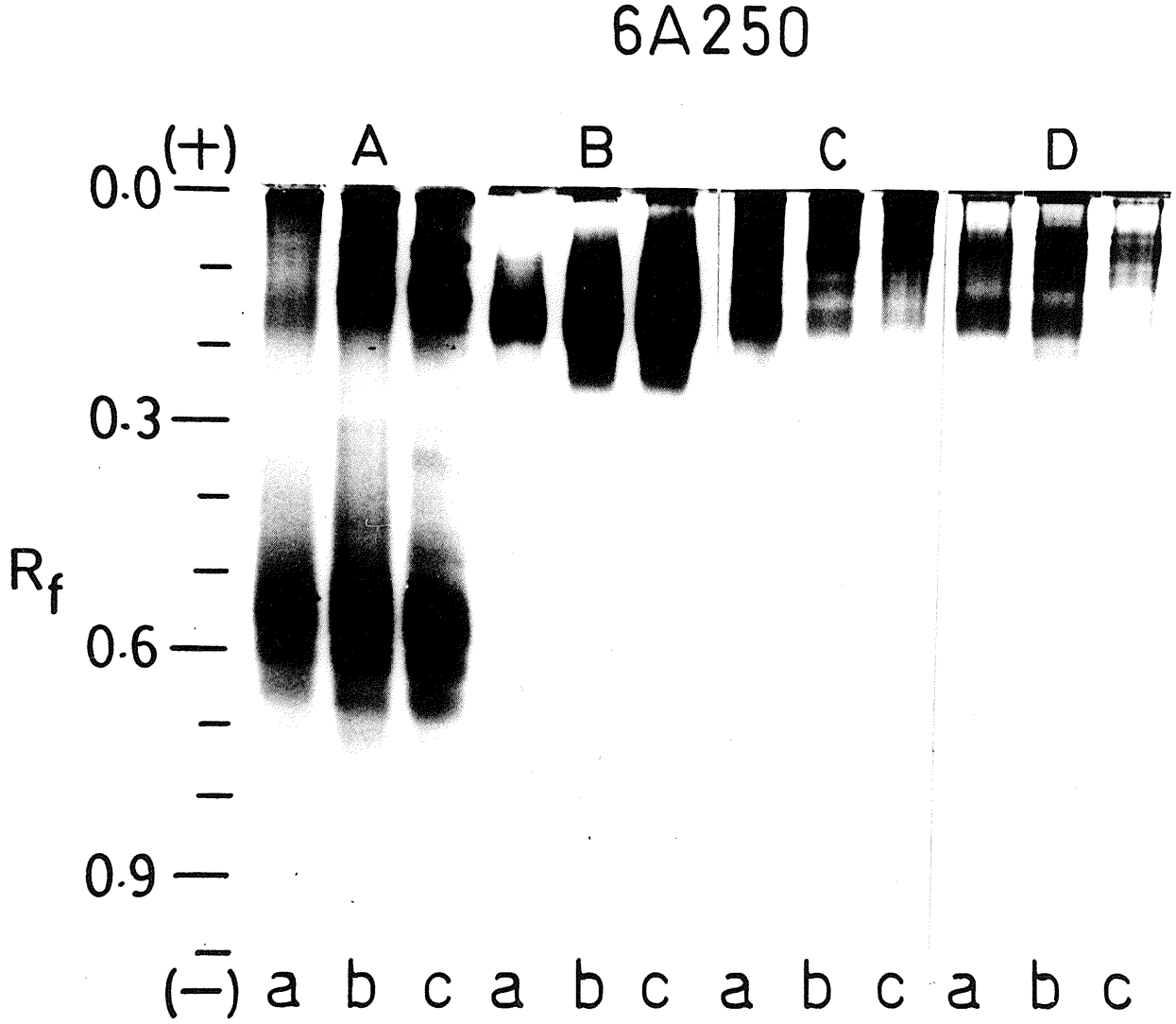

6A250
(+)
A
B
C
D
0.0
0.3
R_f
0.6
0.9
(−)
a b c a b c a b c a b c

Figure 20. Electrophoretic patterns of the albumin-globulin fraction for mature whole meal samples (I = 60 min and II = 180 min).

A. Triticale 6A250.

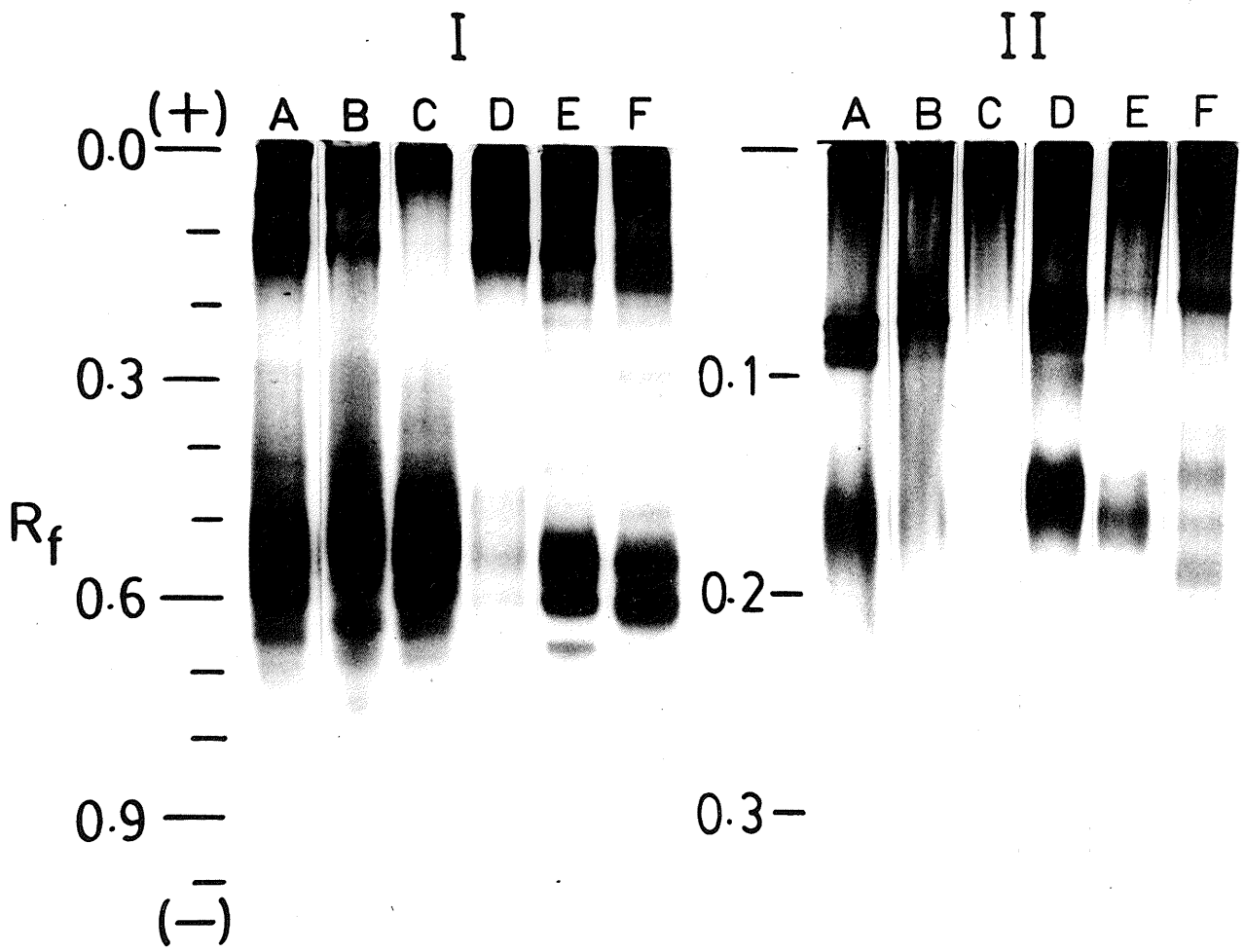
B. Triticale 6517.

C. Triticale 6A190.

D. Prolific spring rye.

E. Stewart 63 durum wheat.

F. Manitou HRS wheat.


I
II
A B C D E F
A B C D E F
(+)
(−)
0.0
0.3
0.6
0.9
0.1
0.2
0.3
R_f

six cereals. For example, the HRS wheat pattern showed at least 26 individual components (the largest number of all cereals examined) whereas the triticale 6A190 contained the smallest number of components (17). It is difficult to discern all of these components from the photograph (Fig. 20-I) because of the low intensity of a considerable number of the bands, especially of those within the R_f range between 0.2 and 0.5. The patterns of the higher R_f region (i.e. $R_f > 0.5$) are similar for all cereals and only small differences in staining intensity are discernable in this region. The greatest variability in the patterns for the various cereals occurred in the low mobility region (R_f from 0 to 0.2). These differences are quite obvious from Fig. 20-II which shows the results obtained with the longer separation time. The mobilities of these slow moving components are similar to those of gliadin components (see later). However, since this extract was obtained with 0.5M sodium chloride solution, it is unlikely that these components are gliadins which are insoluble in this solvent. Varietal and species differences among the six cereals are evident from the patterns in Fig. 20, especially in the low mobility region.

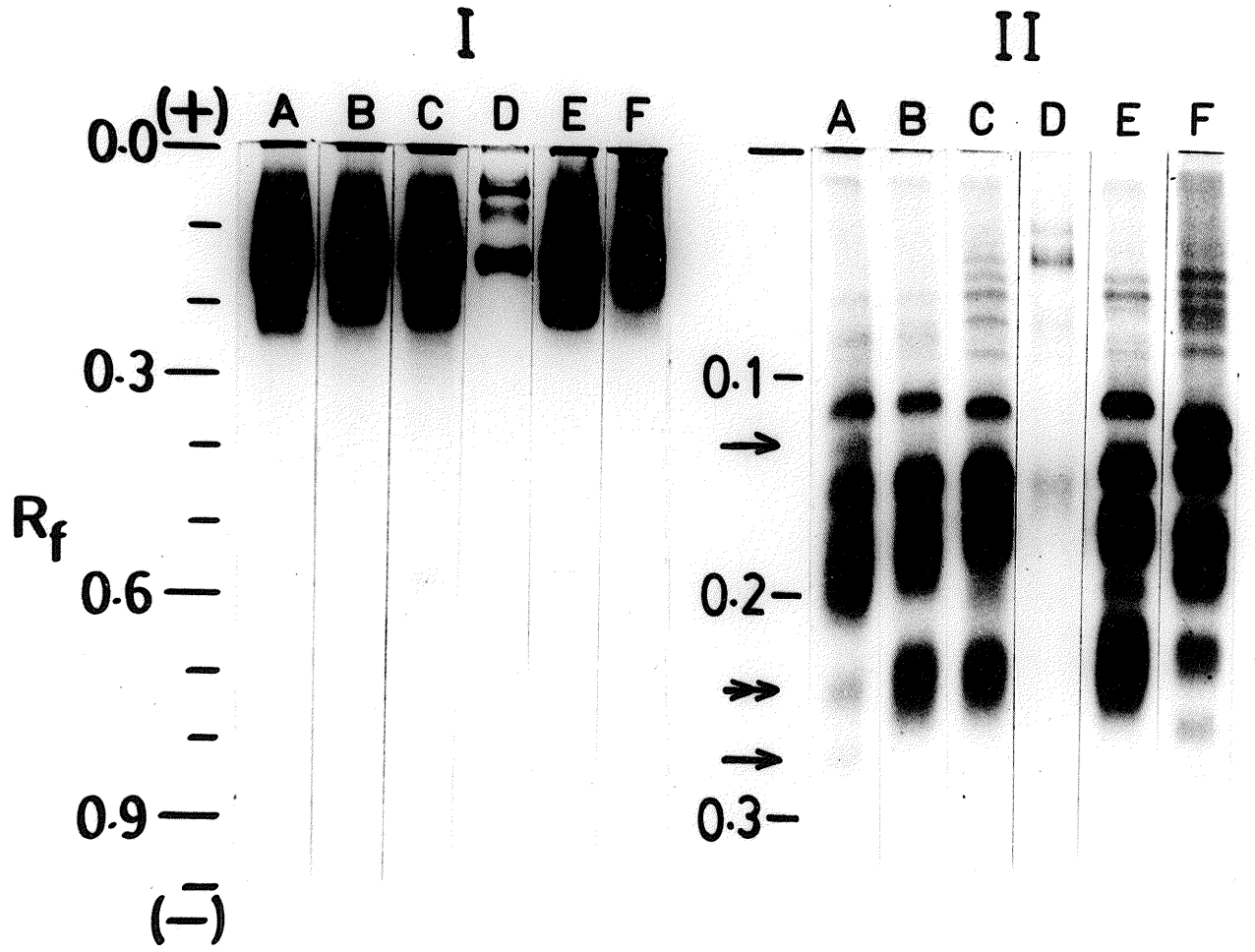
For the three triticales, the albumin-globulin components with R_f's greater than 0.2 are similar and only small quantitative differences are evident. The intensity (concentration) of the slow moving ($R_f < 0.2$) bands showed great variation among the triticales. These slow moving bands were absent in the pattern for 6A190, the most highly shrivelled line. The patterns for the other two triticales in low mobility region were similar, but pattern for the plump line

(6A250) showed that this tline contained a relatively higher concentration of the proteins with R_f's less than 0.2. Thus, as the degree of shrivelling in the triticales increased, the number and the concentration of the slow moving albumin-globulin components decreased quite markedly. Since the albumin-globulin fraction contains metabolically active proteins (enzymes), the missing (or diluted) components may be directly involved in kernel shrivelling. It would be of interest to examine if these components represent specific enzymes. (This was not done in the present study.)

Figure 21 shows the patterns of the gliadin fractions of mature grain of the six cereal species used in this study. The pattern for the rye extract showed the smallest number of components compared with the other cereals. Differences are obvious among the cereals in both the slow ($R_f < 0.1$) and the fast (R_f between 0.1 and 0.3) moving gliadin components. As found by others, the electrophoretic patterns of the gliadins appear to be species (or variety within a species) specific. The fast moving components constitute the major proportion of the gliadin fraction (in terms of relative staining intensity). The gliadin patterns showed some minor bands of high mobility; it is assumed that these are albumin-globulin contaminants.

The patterns of the gliadins of three triticales showed definite differences. The major difference between the patterns of the plump and the shrivelled triticales is in three fast moving components (marked with arrows). The patterns of the shrivelled triticales (6A190 and 6517) do not show two of these three gliadins (marked by a single arrow in Fig. 21-II). The third gliadin component (marked by a double

Figure 21. Electrophoretic patterns of the gliadin fraction for mature whole meal samples (I = 60 min and II - 180 min). Identity of patterns A to F is the same as in Fig. 20.


I
II
A B C D E F
A B C D E F
(+)
0.0
0.3
0.6
0.9
(−)
R_f
0.1
0.2
0.3

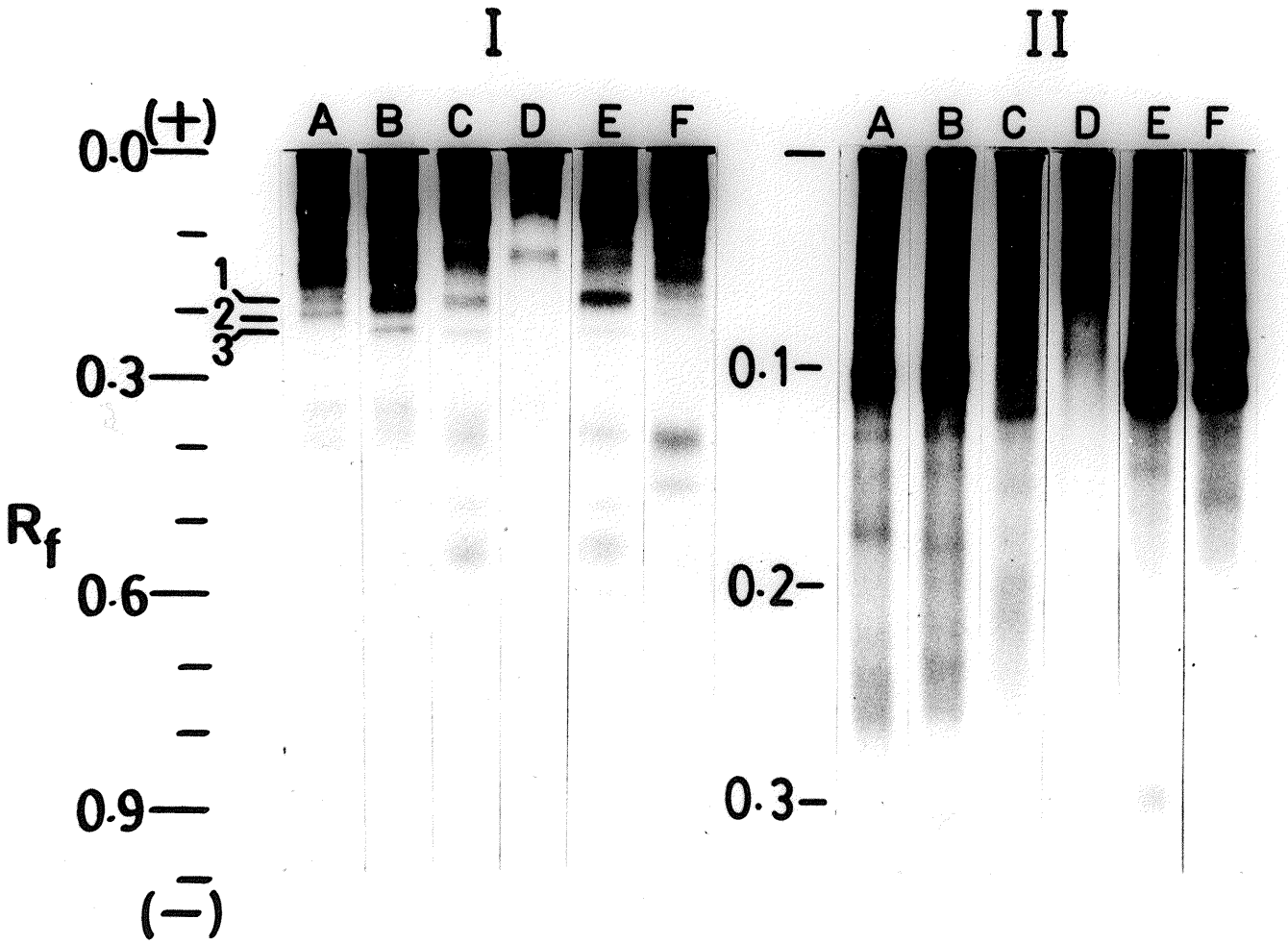
arrow) stained less intensely in the patterns for the plump triticale 6A250. Since differences in gliadin components reflect genetic differences, it is possible that the gene(s) that code the synthesis of the missing gliadin components are linked with gene(s) involved with grain shrivelling.

The electrophoretic patterns of the acetic acid-soluble glutenins are shown in Fig. 22. Although it is not apparent from the photograph, a large proportion of this fraction did not penetrate the gel, but remained at the origin. The proteins that did enter the gel can be separated into a fast moving group ($R_f > 0.3$) and a slow moving group (R_f between 0 and 0.3).

The nature of the fast moving glutenins has not been investigated previously. Whenever they were observed, they were explained as being albumin and globulin contaminants. Results of the present study are in general agreement with this explanation. Accordingly, these fast moving components will not be discussed further in the context of this thesis.

The patterns of the slow moving components of the acetic acid-soluble fractions are similar for all cereals examined, except that the rye pattern showed fewer components. The major differences among the various cereals were in components 1, 2 and 3 (Fig. 22-I). Rye lacked all three of these components; the HRS wheat and triticale 6A250 (plump-grained line) lacked component 3 and the two shrivelled triticales and the durum wheat lacked component 2. In addition, the stain intensity of component 1 was much lower in the HRS wheat and triticale 6A250 patterns than in the other three cereals.

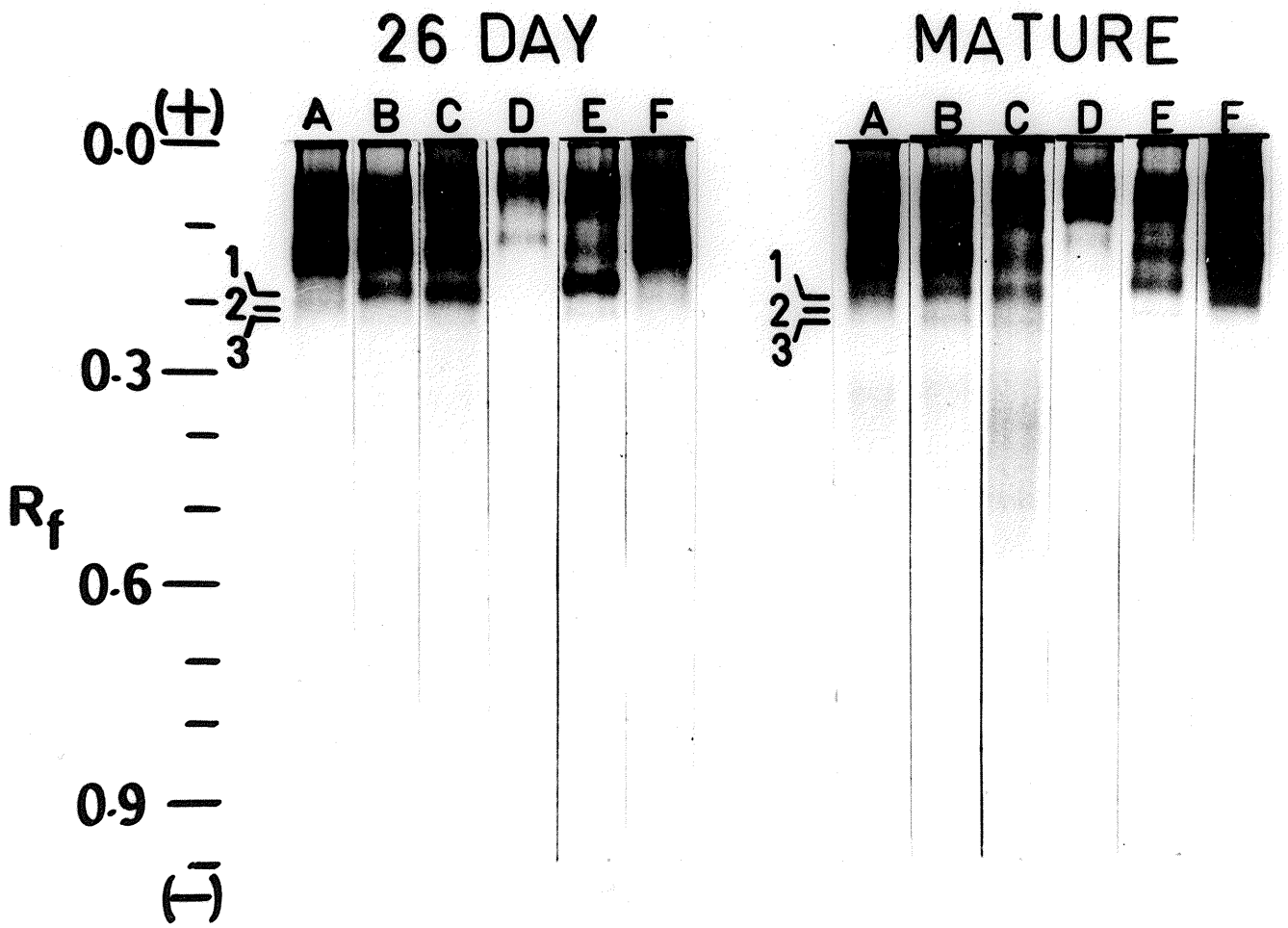
Figure 22. Electrophoretic patterns of the acetic acid-soluble glutenin fraction for mature whole meal samples (I = 60 min and II = 180 min). Identity of patterns A to F is the same as in Fig. 20.


I
II
A B C D E F
A B C D E F
0.0 (+)
0.3
0.6
0.9
(−)
R_f
1
2
3
0.1
0.2
0.3

The electrophoretic patterns of the alkali soluble-proteins are shown in Fig. 23. These patterns are essentially identical to the patterns of the acetic acid-soluble glutenin except that the intensities of the fast moving components are much lower. The major differences among the samples were in the three components designated as 1, 2 and 3 in Fig. 23. These three components are probably the same as the three numbered components in Fig. 22-I. Fig. 23 also includes the patterns of extracts from the 26-day grain samples. These were included to demonstrate the large difference in the band intensity of component 1 among the three triticales. In the pattern for the plump triticale (6A250) component 1 was absent, whereas in the pattern for the two shrivelled triticales it was very prominent.

The examination of the various protein fractions by electrophoresis has shown definite quantitative and qualitative differences between shrivelled and plump triticales. These experiments should be applied to a greater number of triticale lines that show different degrees of kernel shrivelling before it is recommended to the plant breeders as a technique for screening triticale lines for kernel shrivelling.

Figure 23. Electrophoretic patterns of the alkali-soluble fraction for immature and mature whole meal samples. (Electrophoresis was carried out for 60 min.) Identity of patterns A to F is the same as in Fig. 20.


26 DAY
MATURE
A B C D E F
A B C D E F
(+)
0.0
0.3
0.6
0.9
(−)
R_f
1
2
3
1
2
3

VI. GENERAL DISCUSSION

One of the major problems encountered during the development of triticale into a commercial crop is the shrivelled characteristic of its mature grain. The severity of this defect can vary from extensive to minor shrivelling among the various triticale strains that have been examined. Presently, kernel shrivelling is the major factor responsible for the low yield and poor milling quality of triticale grain. Accordingly, the present study was undertaken to extend the pioneering work of Klassen (1970), who examined α-amylase levels and the major carbohydrates in relation to kernel shrivelling in triticale, to the protein components. Since proteins are a more direct expression of genetic information than the carbohydrate components various protein extracts of grain were examined at different stages of grain development. Three triticale strains (6A190, 6517 and 6A250) that exhibit various degrees of kernel shrivelling were used in the present study. In addition, the study included one cultivar of rye, durum wheat and bread wheat for comparison purposes. It was anticipated that the proteins (including the enzymes), during early kernel development, would reflect more accurately differences between triticale strains that yield shrivelled and plump grain and consequently present a better insight into the cause(s) of kernel shrivelling.

Pertinent differences and similarities among the six cereals were discussed in the Results and Discussion section and will not be considered further. This section of the thesis will emphasize only the differences in the various protein and carbohydrate components in

relation to kernel shrivelling in triticale, in keeping with the prime objective of the thesis project.

Grain of the shrivelled-grained triticale strains generally have a higher frequency of aneuploidy than grain of plump-grained triticale strains and this is especially true for triticale 6A190 (Larter, personal communication). With the high frequency of aneuploidy and the extremely high α-amylase activity in mature grains of triticale 6A190, this particular strain is not representative of an average triticale and is therefore rather unique among the more successful triticales in this respect. However, because of the severely shrivelled nature of its mature grain, triticale 6A190 was included in the study to represent the extreme of this kernel defect. Triticale 6517, the strain that yields grain with intermediate degree of shrivelling, generally had a developmental pattern for a particular chemical component that was intermediate in the patterns for triticales 6A190 and 6A250 (a strain with relatively plump grain). Frequently the developmental patterns or values for a particular component of the two triticales that yield shrivelled grain (6A190 and 6517) were similar. Accordingly, the discussion of the results, presented here will emphasize the differences between the two shrivelled- and plump-grained triticales.

Throughout kernel development, grains of the shrivelled triticales had a significantly higher nitrogen content than the grain of plump triticale 6A250 (Fig. 3). Comparison of the developmental pattern for total nitrogen on a per kernel per cm^3 and weight basis suggests that the higher nitrogen level in the shrivelled triticales is probably due to reduced synthesis of other grain components such as starch. For

triticale 6A250 the decrease in nitrogen content, between 12 and 22 days after anthesis, coincides with the time of rapid starch synthesis. The absence of analogous decrease in nitrogen content in the shrivelled triticales indicates that the relative rate of protein synthesis is faster in these two triticales than in the plump triticale 6A250. Conversely, the rate of starch synthesis is faster in triticale 6A250 than in the shrivelled triticales 6A190 and 6517. Thus, variations in nitrogen (protein) content, during kernel development, reflect metabolic differences between shrivelled and plump triticales in both protein and starch synthesis.

Reducing sugars content of the three triticales showed no significant differences, during kernel development, between shrivelled- and plump-grained strains. At maturity, the shrivelled triticales had a slightly higher content than the plump triticale 6A250.

On the other hand, shrivelled- and plump-grained triticales can be distinguished on the basis of the non-reducing sugars content of their immature grain. The shrivelled triticales 6A190 and 6517 had significantly higher amounts of non-reducing sugars, during early development, but by maturity the difference was not significant with the experimental error of the analysis.

The developmental patterns for both reducing and non-reducing sugars showed a decreasing trend during kernel development in the three triticales (Figs. 4 and 5). Obviously, the maturing kernel utilizes these sugars for starch synthesis and/or other metabolic functions. The lower non-reducing sugars content of the immature grain of triticale 6A250 (plump) indicates that the utilization of these metabolites is more

efficient in the plump triticale than in the shrivelled triticales. Differentiation of the shrivelled- and plump-grained triticale strains on the basis of non-reducing sugars content of immature grain is possible, however it might be rather uncertain because of the difficulty of selecting immature grain of equal physiological maturity.

The three triticales examined have similar starch developmental patterns, in that starch content increased during kernel development. However, starch content of the plump triticale 6A250 is considerably higher, throughout development, than for the shrivelled triticales (Fig. 6). The results of the present study support the earlier suggestion of Klassen *et al.* (1971) that triticale ceases starch synthesis at an earlier stage in development than wheat and that shrivelled-grain triticale strains cease starch synthesis earlier than plump-grained triticale strains. The findings of this study extend the abnormal-starch synthesis idea further, as it indicates that the starch synthesizing apparatus in the shrivelled triticales 6A190 and 6517 is much slower (or less effective), from the onset of synthesis, than in the plump triticale. Thus, the major factor responsible for the difference in starch content of grain, between shrivelled and plump triticales appears to be established during the early stages of kernel development.

To determine whether the lower starch content of the grains of shrivelled triticales is due to a specific component of starch, amylose content was determined for different stages of kernel development. The amylose developmental patterns were similar to the starch patterns; shrivelled triticales had lower amylose content than the plump triticale 6A250. Comparison of the two starch components, on an amylose to amylopectin ratio basis, reflects a higher rate of amylose synthesis in the

plump triticale than in the shrivelled triticales 6A190 and 6517 (Table 5). However, the difference in amylose content among the triticales does not account for all the difference in starch content between shrivelled and plump triticales; consequently both starch components (amylose and amylopectin) contribute to this difference. Both starch and amylose contents can be used to distinguish between shrivelled- and plump-grained triticale strains used in the present study.

The shrivelled triticales 6A190 and 6517 were characterized by having higher α-amylase levels, both during kernel development and at maturity, than that of the plump triticale (Fig. 8). The two shrivelled triticales showed a large increase in α-amylase activity from the 26-days after anthesis to maturity. Triticale 6A250 showed no such increase in activity; actually it showed a slight decrease. The higher α-amylase activity of the shrivelled triticales was not reflected in higher reducing sugars levels, however at early stages of kernel development non-reducing sugars contents may be a reflection of the higher α-amylase activity.

To explain the higher α-amylase activity in the shrivelled triticales, the α-amylase isoenzymes were examined during kernel development and germination. α-amylase isoenzymes were classified into two distinct groups according to their isoelectric points (Fig. 9). The activity (intensity of isoenzyme bands) of the low IpH α-amylases showed a decreasing trend with maturation for the plump triticale 6A250. The activities of the low IpH isoenzymes for the two shrivelled triticales were similar in 26-day and mature samples (i.e. did not show the decreasing trend of the plump triticale). The activity patterns of the high IpH α-amylases showed similar trends with development as the low IpH isoenzymes for both

types of triticale. Since the isoenzyme patterns of germinated grain were different from the patterns of mature grain, the high activity in the mature grains of the shrivelled triticales, was attributed to a lack of suppressing mechanism or presence of activators (acting on existing α-amylases) but does not necessarily rule out the involvement of germination process. Both the α-amylase activity and the isoenzyme band intensity could be used quite accurately to distinguish between the shrivelled- and plump-grained triticale strains. Accordingly, these characteristics can be used for selecting lines of particular kernel characteristics in breeding programs.

Immature grain of the three triticales had similar proteolytic activity, whereas at maturity the shrivelled triticales 6A190 and 6517 had significantly higher activity than the plump triticale (Fig. 10). Accordingly, the proteolytic isoenzymes were examined during development and germination to obtain further information on this enzyme in relation to kernel shrivelling.

Four isoenzymes were detected in each triticale; all isoenzymes decreased in activity (band intensity) with maturation. There were no distinguishable differences in the proteolytic isoenzyme patterns from grain of shrivelled and plump triticales nor between mature and germinated grain (Fig. 11).

The three triticales showed significant differences in peroxidase activity at various stages of kernel development. At 14 and 18 days after anthesis, the activity paralleled the degree of kernel shrivelling. However, this trend reversed at day 26, when the plump triticale 6A250 had the highest activity. At maturity the activity once again was highest in the shrivelled triticales.

This cross-over pattern in peroxidase activity between shrivelled and plump triticales warranted an examination of its isoenzyme patterns during kernel development which was carried out. The anionic peroxidase isoenzymes (Fig. 13A) showed a general decrease in activity (band intensity) with maturation, whereas the cationic isoenzymes (Fig. 13B) showed an increasing trend. The isoenzyme pattern (both anionic and cationic) of the severely shrivelled triticale 6A190 differed considerably from the other two triticales both in band intensity and in the number of bands. The triticale of intermediate degree of shrivelling and the plump triticale had similar patterns throughout kernel development. However, the plump triticale 6A250 appeared to have more intense cationic bands in the 12- and 26-day samples than the two shrivilled triticales. One peroxidase isoenzyme (No. 19 - Fig. 13B) was present in the germinated grain of all three triticale, but this particular isoenzyme was only evident in immature and mature grains of the plump triticale strain. Consequently, the presence or absence of isoenzyme 19 in developing grain can distinguish between shrivelled and plump triticales used in this investigation. Because the functional roles of specific peroxidase isoenzymes in the developing kernel are not known, it is not possible to speculate on the implications of the presence or absence of a specific peroxidase in the metabolism of the grain.

The shrivelled triticales 6A190 and 6517 differed significantly from the plump triticale in total nitrogen (and presumably protein) content during kernel development. Accordingly, possible differences in molecular weight distribution of the proteins were examined by gel filtration (Fig. 15) and SDS-PAGE (Figs. 16 and 17). The gel (Sephadex G-150)

elution profiles revealed both qualitative and quantitative differences, during development, in the proteins. In mature grain, the shrivelled triticales had lower amounts of the high MW proteins (peaks I and II; Table 6) and higher amounts of the low MW proteins (peaks IV and V) than the plump triticale. These differences between the low and high MW components reflect different metabolic rates in the synthesis of these two classes of proteins in shrivelled- and plump-grained triticale strains. This is most evident when changes in peak V (Table 7) are compared for the 12-day and mature samples. However, the elution profiles of gel permeation chromatography cannot distinguish easily between shrivelled and plump triticales, although differences can be observed.

The presence of more high MW proteins in the plump triticale 6A250 may be significant to its utilization, especially for human usage in leavened baked products. These proteins play a key functional role in the breadmaking quality of wheat (Pomeranz, 1971), and presumably would be equally important for triticale.

SDS-PAGE patterns of reduced and non-reduced proteins revealed both quantitative and qualitative differences among the triticales. However, no significant difference was observed in the patterns of triticale strains that yield shrivelled and plump seed, except that the 72,000 subunit (marked by double arrow in Fig. 17) was more prominent in the mature whole meal and flour samples of the shrivelled triticales 6A190 and 6517.

Since differences in the molecular weight distribution of proteins, during kernel development, in grain of shrivelled and plump triticales were apparent, the proteins were further examined by the modified Osborne

solubility fractionation procedure. The shrivelled triticales had the highest amounts of non-protein nitrogen throughout kernel development. This difference suggests a more efficient utilization of these nitrogenous components in the plump triticale 6A250.

Only minor differences in the albumin-globulin fractions were apparent between triticale strains that yield shrivelled and plump grain. The plump triticale 6A250 had slightly more of this fraction throughout kernel development than the shrivelled triticales. In addition, the percentage of this fraction at maturity was somewhat lower in the non-endosperm grain components of shrivelled triticales (Table 8). Since the albumins and globulins are the most metabolically active proteins, even small differences in this fraction may be related to the shrivelling problem.

No significant difference was found in the gliadin fraction of grain for shrivelled and plump triticales.

The shrivelled triticales contained more glutenin at maturity than the plump triticale 6A250. However, when the glutenin and alkali-soluble fractions are combined (Table 9), no significant differences were apparent between triticale strains that yield shrivelled and plump grain.

Since the protein solubility fractionation results revealed only small differences between shrivelled and plump triticales each solubility fraction was examined further by polyacrylamide gel electrophoresis. Examination of equivalent protein fractions revealed major differences that may be related to kernel shrivelling in triticale. These are discussed below.

Among the albumin-globulin proteins, the slow moving components (with

R_f's < 0.2; Fig. 20) showed the greatest variation among the triticales. The plump triticale 6A250 had a high concentration of these components. For the two shrivelled triticales, the concentration was relatively low in 6517 (compared to 6A250), whereas in 6A190 these slow moving components could not be detected. Other minor quantitative differences were observed in the albumin-globulin fractions of triticales, but these differences do not appear to be related to grain shrivelling.

The gliadin electrophoretic patterns of triticales showed that three fast moving components (marked with arrows in Fig. 21-II), could differentiate shrivelled and plump triticales. The shrivelled triticales lacked the fastest and the slowest of these components, whereas the concentration of the third component was much lower in the plump triticale 6A250. Since gliadin proteins can be used as genetic markers, it is possible that the gene(s) involved in the synthesis of these three components may be somehow related to kernel shrivelling. This, at least, appears to be the case for the three triticales selected for this study.

The electrophoretic patterns of the glutenin and alkali-soluble proteins for the three triticales were similar. However, three components, which appear to be identical in both fractions, could clearly distinguish between shrivelled- and plump-grained triticale strains (Figs. 22 and 23). Component 1 was present in much greater quantity (more intense bands) in the shrivelled triticales than in the plump triticale 6A250. The shrivelled triticales 6A190 and 6517 lacked component 2, whereas the plump triticale lacked component 3.

In summary, the preceding paragraphs attempted to emphasize differences, in the protein and carbohydrate components of grain, between

triticale strains that yield shrivelled and plump grain. Because of the exploratory nature of this work, these observations should be extended to a wider range of triticales in order to disclose which differences are truly characteristic of shrivelled or plump grain. Only then can one confidently attempt to interpret the biochemical basis of these differences.

Further biochemical investigations are obviously required to explain the nature and causes of kernel shrivelling. Such work might examine some of the protein components, which differed in shrivelled and plump grains, for specific enzyme activity. Also, the starch synthesizing apparatus should be examined in greater detail, especially during early kernel development. Marker proteins or enzymes (that are present only in shrivelled or plump grain) could be used as the screening method in the selection for plump grain and in a genetic search for parents for new triticale strains. The study of specific hormones, activators and inhibitors of enzymes, might present some information on the roles of these compounds on specific enzymes, such as the amylases and peroxidases, during kernel development. These research areas could well form another thesis project.

VII. CONTRIBUTIONS TO KNOWLEDGE

The protein (including enzyme) and carbohydrate components of grain for the three triticale strains (that vary in the degree of kernel shrivelling), spring rye and durum wheat parents of triticale 6A190 and a HRS wheat were compared at various stages of kernel development. Definite differences existed in the protein, enzyme and carbohydrate components of grain from shrivelled- and plump-grained triticale strains. The more distinct contributions of this investigation are outlined below.

1. The developmental patterns of non-reducing sugars and amylose contents for triticales were established and compared to the patterns for rye and wheat.
2. Two distinct groups of α-amylases were found in immature and mature grains of triticale as determined by isoelectric focusing.
3. Developmental patterns for proteolytic and peroxidase activities were established for triticale and compared to the patterns for rye and wheat.
4. Four proteolytic isoenzymes were detected in each cereal species and each isoenzyme decreased in activity with grain maturation.
5. Peroxidase isoenzyme developmental patterns of the triticales were compared to each other and to the patterns for rye and wheat.
6. One peroxidase isoenzyme appeared in immature and mature grain of only the plump triticale.
7. Two catalase isoenzymes were detected in all cereal species.

8. The gel filtration profiles of grain protein for the three triticales were compared to each other and to the profiles for rye and wheat at the various stages of kernel development.
9. The non-reduced and reduced SDS-PAGE patterns of an AUC protein extract were compared for the six cereals.
10. The nitrogen solubility distribution of grain for the three triticales, at various stages of development and of flour, were compared.
11. Each of the four protein solubility fractions reflected specific protein components that could distinguish between shrivelled- and plump-grained triticale strains.

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