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

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

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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.

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# 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