AN INVESTIGATION OF KERNEL SHRIVELLING IN TRITICALE

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by

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ABSTRACT

Triticale lines selected for variation in degree of kernel shrivelling were studied to test the hypothesis that shrivelled kernels were the result of the rapid breakdown of endosperm starch to sugars following initiation of premature germination processes.

Grain density measurements were highly correlated with bushel weight and also agreed well with the visual rating of shrivelling and, thus, were used as the index of degree of kernel shrivelling. Alpha-amylase activity of mature Triticale grain varied greatly between lines and was inversely correlated with grain density. That is, lines with low density had high amylase content. To study the development of the amylase activity a line with low activity (6A320) and a line with high activity (6A190) were compared at different stages of grain maturity. In both lines amylase activity was high at 10 days followed by a rapid decrease to 38 days at which time activity in 6A190 increased very rapidly as compared to only a slight increase in 6A320.

As expected, reducing sugar content of mature grain reflected the alpha-amylase activities. In addition, developmental patterns of reducing sugars coincided closely with the alpha-amylase patterns for the lines 6A190 and 6A320. Reducing sugar levels in Manitou wheat decreased steadily during early stages and reached a constant low level by maturity.

Starch content at maturity was not correlated with either alpha-amylase or reducing sugars but a correlation of 0.75* was obtained for starch content and grain density. Starch accumulation profiles during grain development were similar for 6531, 6A320 and 6A190 at early stages, however, at approximately 55 percent moisture starch deposition in 6A190

ceased while 6531 and 6A320 continued to increase. Consequently, at maturity 6A320 and 6531 had about 6 and 10 mg. more starch per kernel respectively than 6A190. In Manitou deposition continued until approximately 40 percent moisture. These patterns were very similar to the patterns of dry matter accumulation.

Mean endosperm starch granule size of Triticale lines ranged from 17.81 to 25.61 μ as compared to 18.70 for Manitou and 28.24 for Prolific rye. There was no consistent relationship between starch granule size and degree of kernel shrivelling. The three Triticale lines with the lowest mean starch granule size all had Triticum persicum included in their parentage.

Grains of 16-day old excised heads of 6531 incorporated approximately 10 percent more sucrose-^{14}C into endosperm than 6A190 indicating that the rate of synthesis in endosperm tissue of 6A190 is probably lower than 6531.

No improvement in grain development was observed following the injection of 1 ml. of 10^{-1}M CCC into the culms of 6A190 and 6531 either at flowering or 31 days later. Alpha-amylase activity in CCC treated grains of 6A190, however, was reduced to 78 percent of the untreated controls suggesting that kernel shrivelling cannot be directly attributed to high levels of alpha-amylase.

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1. INTRODUCTION

Artificially synthesized interspecific and intergeneric plant hybrids have been extensively employed to study genetic relationships among species and for this purpose they have proved to be extremely useful. More recently they have also been used as tools in the transfer of desirable genes into cultivated crop species. Triticale, which is a combination of Triticum (wheat) and Secale (rye) genomes, has for some time now received close attention as a potentially useful commercial crop. A comprehensive discussion of the breeding program and basic research at the University of Manitoba directed toward the development of Triticale as a commercial crop from 1954 to the present may be found in a paper by Larter (1968).

In order to develop wheat-rye hybrids to a point where they possess desirable levels of agronomic and quality characteristics a number of problems must be overcome. While a great deal of progress toward improvement has been made several areas need further attention.

One of the persisting problems in the development of Triticale is its poor kernel characteristics. The kernels, rather than being well filled and plump like wheat, are severely shrivelled at maturity, thus resulting in a low weight per bushel. This shrivelling is independent of environment and occurs even under favourable growing conditions (personal observation).

It had been frequently observed that Triticale was more susceptible to germination in the ear prior to or at maturity than locally adapted cereal varieties. In addition, reports from a number of sources indicated that mature Triticale grain was higher in alpha-amylase activity than sound wheat (see for example, Müntzing, 1963).

In view of these findings and observations it was postulated that shrinkage and partial collapse of the endosperm of Triticale might result from the rapid conversion of starch to sugar associated with the onset of precocious germination.

The objectives of this study were to test this hypothesis by determining the nature of the relationships among kernel shrivelling, alpha-amylase activity, and carbohydrate content of Triticale lines.

2. LITERATURE REVIEW

2.1 Breeding and development of Triticale

Triticale is an allopolyploid produced by doubling the chromosome number of the sterile hybrid that results from a cross between wheat, Triticum aestivum L. em Thell (group aestivum) or T. turgidum L. (group durum) and rye, Secale cereale L.

Literature relating to the breeding and development of wheat-rye hybrids from the earliest reports in the later 1800's to the present has recently been reviewed by Briggle (1969).

The first serious breeding work with Triticale was begun in Sweden in 1934 by Müntzing (1939). Working with octaploid (56 chromosome) Triticales he found that all lines were partially sterile and pollen fertility was lower than in standard wheat varieties. Spanish workers headed by Sanchez-Monge (1955) also investigated the usefulness of 56-chromosome Triticales but found that shrivelled grain and sterility were problems. This led him to speculate that 42 chromosomes would be nearer the optimum number for Triticale than 56 chromosomes. However, he found that the grain of hexaploid Triticales resembled that of the octaploid Triticales in that it was shrivelled and of low quality resulting in low flour yields, (Sanchez-Monge, 1958). Using gamma irradiation of the female parent immediately after emasculation followed by pollination with pollen of sister plants Sanchez-Monge (1968) was able to achieve some improvement in endosperm quality. From 77 progenies it was possible to select 5 with smoother seeds. Reciprocal crosses between these 5 lines and their original lines seemed to indicate a plasmagenic influence on endosperm quality.

Canadian Triticale research initiated in 1954 has also been concentrated on the improvement of 42 chromosome types. Considerable progress has been made in the improvement of agronomic characteristics such as plant height and maturity (Larter, et al., 1968). Partial sterility and shrivelling of the grain are limiting factors at present. Cytological instability which exists within Triticale lines is believed to be the cause of the partial sterility. Aneuploids were observed with a frequency of 10-15 percent in a routine somatic chromosome count of bulk seed samples from 30 strains. The majority of the aneuploid types were hypoploid. They observed that any one of the species Triticum timopheevi, T. persicum or Secale montanum, when included in the parentage of a Triticale hybrid, contributed genes for desirable kernel characteristics to the progeny.

2.2 Alpha-amylase development

Schwimmer (1947) found that the amount of alpha-amylase per wheat kernel remained relatively constant throughout the entire course of development and early stages of ripening. However, on a per gram of dry matter basis immature wheat had about 68 times the activity of mature grain. In contrast, the amount of beta-amylase per gram dry weight increased with the development of the grain, being attended at the same time by a decreased solubility in water. Similar results were reported by Olered (1964), namely that alpha-amylase activity per unit of dry matter in wheat was very high during the first stages of kernel development and that there was then a very rapid reduction such that when the grain had reached the "milky stage" the residual alpha-amylase activity was already very small.

Initiation of germination processes in cereal grains are characterized by large increases in alpha-amylase activity. In rye with as little as 0.5 percent visibly germinated seed the amylase value was nearly always high (Tedin and Persson, 1963). Even among the plants with no visibly germinated seeds there were many with very high amylase activity. Likewise, in winter wheat Bingham and Whitmore (1966) observed that there were considerable varietal differences in susceptibility to germination in the ear and such germination was always associated with an increase in alpha-amylase activity. However, even in the absence of germination some varieties exhibited high levels of alpha-amylase and the authors concluded that alpha-amylase activity appeared to be under two types of genetic control. From the point of view of plant breeding it is thus not sufficient only to select against visible sprouting but it is necessary to carry out chemical assays for amylase activity as well. Selection against alpha-amylase activity in rye was shown to be effective.

Müntzing (1963) noted that rye had a stronger tendency to pregermination than wheat and that in octaploid wheat-rye hybrids this tendency seemed to be just as marked as in rye. Variation in alpha-amylase activity was found among different lines of Triticale and the line with the lowest amylase activity was also the one with the best kernel type as determined by visual judgement.

In germinating rice seeds rapid breakdown of endosperm starch began after about four days of germination (Murata, et al., 1968). Alpha-amylase activities were found to parallel the pattern of starch breakdown. Juliano and Varner (1969) studying germinating peas found that starch was degraded slowly in the first 6 days, a period during which alpha-amylase activity was very low. Beta-amylase was present at a

constant level while phosphorylase gradually increased and reached a peak on the fifth day. Beginning on the sixth day there was a more rapid degradation of starch which coincided with alpha-amylase production. The authors consequently concluded that alpha-amylase was the major enzyme involved in the initial degradation of starch into more soluble forms while phosphorylase and beta-amylase assisted in the further conversion to free sugars.

2.3 Gibberellins and amylase activity

The stimulation by gibberellic acid (GA) of alpha-amylase activity and consequently of sugar release from starchy endosperm is well documented and is being utilized on a commercial basis in the malting industry.

Paleg (1960) was one of the first to demonstrate that added GA stimulated the production of amylase enzyme and release of reducing sugars from barley endosperm. Yomo (1958) found that separated barley embryos and endosperms produced less alpha-amylase when cultured separately than when cultured together on a gel of agar. He subsequently showed that the embryo produced a gibberellin-like hormone which diffused from the scutellum and activated the endosperm to produce hydrolytic enzymes, which then in turn degraded the endosperm. When treated with GA, cell walls of barley endosperm first break down at the periphery adjacent to the aleurone layer indicating that the aleurone layer must be the site of GA action (Briggs, 1963). The amino acid analogues DL-ethionine and DL-p-fluorophenylalanine were potent inhibitors of sugar release and also depressed the incorporation of radioactive amino acids into soluble protein. Since this phenomenon is a well known aspect of the inhibition of de novo protein synthesis, Briggs concluded that GA acts by stimulating

de novo enzyme synthesis rather than by activating a preformed enzyme. These observations and conclusions were supported by the work of Varner (1964). Visual inspection of GA-treated barley half-seeds revealed that that part of the starchy endosperm in contact with the aleurone layers was first dissolved indicating that the alpha-amylase was probably coming from the aleurone layer. This was confirmed quantitatively. Following incubation with phenylalanine-^{14}C alpha-amylase was found to be labelled and to constitute a major fraction of the label incorporated into protein. The author questioned whether the entire amylase molecule was produced by de novo synthesis or whether the labelling resulted from addition to or modification of a precursor as is the case in B. subtilis. Using density labelling of barley alpha-amylase with H_2O^{18} Filner and Varner (1967) showed that all of the enzyme was synthesized de novo in response to gibberellic acid.

Radley (1967) showed the scutellum to be the site of production of gibberellins in the early stages of the germination of barley seed. Activity of the scutellum ceased on the third day at which time the axis probably commenced to produce gibberellins. CCC (2-chloroethyl trimethylammonium chloride) was an effective inhibitor of gibberellin production.

All concentrations of GA_3 (0.07-0.09 µmoles/plant) sprayed onto intact leaves of tobacco plants caused a significant reduction of starch levels in the leaves (Lee and Rosa, 1969). Reducing sugar levels increased but were not great enough to account entirely for the loss of starch in GA_3 treated leaves. GA_3 treatment also resulted in a significant increase in amylase activity and the authors attributed the decrease in starch content to this enzyme stimulation.