

ASPECTS OF PROTEIN CONTENT
IN SPRING RYE

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of
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James Grant McLeod

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A dissertation submitted to the Faculty of Graduate Studies of
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ABSTRACT

McLeod, James Grant. Ph.D., The University of Manitoba, October, 1978. Aspects of Protein Content in Spring Rye. Major Professor; Dr. E.N. Larter.

This study was conducted in an attempt to define the variation for protein content in inbred lines of spring rye (Secale cereale L.) and to determine the possible use of such variation in the improvement of protein content in spring rye. Analysis of 465 inbred lines of spring rye, derived from various sources and grown in field experiments, revealed considerable variability for protein content in most sources. The variance of several groups of inbreds was similar at different levels of inbreeding. S_3 lines were as differentiated for protein content as were long term inbreds. The possibility of selecting on an S_1 plant basis was also suggested. Mean kernel weights of inbred lines were about 53% of Gazelle, a standard cultivar. Seed weight versus protein percent correlations showed a negative trend, as expected. In 1975 yields of inbred lines were very erratic and comparisons were not made.

Thirty inbred lines were studied in 1976 and 1977 in further detail in 3-replicate single-row tests. They showed a negative trend in the correlations of protein

percent and yield. Correlation of yield versus kernel weight was positive and high. Nine of the 10 highest yielding inbred lines in 1976 were among the 10 highest yielding lines in 1977. A preponderance of low protein inbreds was observed among the 10 highest yielding lines.

Thirty hybrids were synthesized from inbred lines and used to study the inheritance of protein levels in the grain. Inbred lines having low protein levels combined to give hybrids with low protein content in the grain while inbred lines of high protein content combined to give hybrids with high grain protein as well. High protein inbreds when combined with inbreds of low grain protein resulted in hybrids having intermediate grain protein levels.

Heritability as predicted by the regression of the F_1 generation values on mid-parent values was $0.52 \pm .10$ and $0.55 \pm .09$ in 1976 and 1977, respectively. Regression of the F_2 generation on the mid-parent values was $0.51 \pm .08$. Protein percent and protein yield of the F_1 hybrids were positively correlated with grain yield by rank correlation.

Of the 30 F_1 hybrids synthesized, two in 1976 and 13 in 1977 were equal to or greater than Gazelle in yield. Protein percent was not correlated with grain yield or kernel weight. However, protein percent and protein yield as well as protein yield and grain yield were strongly correlated as shown by rank correlation coefficients.

Analysis of a six-parent diallel cross for general combining ability (GCA) and specific combining

ability (SCA) revealed GCA effects to be of greater importance than SCA effects. Therefore, the characters studied appear to be inherited in an additive fashion and should be amenable to direct selection. Protein percent-seed weight regressions of the F_3 lines of the diallel, generally were non-significant. Means of the F_1 , F_2 and F_3 generations were intermediate to the parents only in crosses where the parents represented a wide range of protein levels.

Yield and kernel weight both showed inbreeding depression. Inbreeding depression occurred from the F_1 to F_2 generations for both grain yield and 200-kernel weight in nearly all hybrids. Protein percent showed negative inbreeding depression in a high proportion of the hybrid populations. Protein percent was depressed in most hybrids of the diallel, contrary to expectations. This may have been due to the small sample of inbreds used in the diallel cross.

INTRODUCTION

Many studies have been conducted in the past to define the mode of inheritance and the heritability of protein content in the cereals. In rye, protein is concentrated in the aleurone tissues, therefore, large seeds generally have a lower percentage crude protein content than smaller seeds. However, there are well known examples where this correlation does not exist.

In recent years, qualitative characters which change the quality of the protein in maize (Mertz et al., 1974), in barley (Hagberg and Karlsson, 1969) and in sorghum (Singh and Axtell, 1973), have been transferred into cultivars. In these three particular cases, detrimental effects on the anatomy of the seed and on yield have been observed. Other observations have indicated that these genes shift the distribution of storage proteins from the alcohol soluble prolamin fraction to the acetic acid soluble glutenin fraction by suppression of certain prolamines (Lee et al., 1976), thus improving the quality of the protein in terms of amino acid balance.

The protein of rye has been shown to be superior to that of other cereal grains, both in quantity and quality by several researchers. Values for lysine tend to

be higher for rye than for other cereals. This is probably because rye has proportionately less of its storage protein in the prolamin fraction than does other cereal grains (Chen and Bushuk, 1970).

Plant breeders can design programs of varietal improvement much more easily with a knowledge of the mode of inheritance and heritability of various agronomic traits. However, with the exception of a number of simply inherited and, therefore highly heritable genetic traits in rye, i.e. self-sterility, kernel characters and plant type; little is known about inheritance in rye. Wolski et al., (1972) indicated that yield per spike, yield per plot and straw strength tended to have low heritabilities in open pollinated populations of rye. Wricke (1973) concluded that grain yield in rye had a large dominance component. This suggests that there would be an advantage to commercial hybrids. He did not detect epistatic gene action for grain yield, height or mean kernel weight.

This study is an attempt to provide some knowledge of the heredity of rye protein content, an area in which little is known. It is also an attempt to illucidate further the problem of whether high yield and high protein content can be combined, a relationship still open to question. This study will also provide genetic material for use in triticales programs.

Other questions of plant breeding interest such as the relationship between inbred performance and hybrid

performance for protein content may be answered. The experimental material used in this study consisted of lines of rye made available by the Plant Science Department of the University of Manitoba. The inbreds were developed by inbreeding diverse source populations. To the author's knowledge, no study has to date been attempted in which protein inheritance in rye has been evaluated by the use of such theoretically homozygous inbred lines.

LITERATURE REVIEW

2.1 Composition of the rye kernel

2.1.1 Pericarp

The distribution of protein in the kernel is related to its function in the development and germination of the grain. The pericarp layers of the kernel (outer pericarp, outer and inner integument and nucellar tissue) degenerate during kernel maturation. Approximately four percent of the total protein is in these layers at maturity and is probably complexed with tannins.

2.1.2 Endosperm

The aleurone layers of the endosperm serve as a storage organ for protein. The proteins of the aleurone are readily metabolized and are available to provide a fast source of energy and amino acids for enzyme synthesis in germination. Metabolically active proteins are concerned with kernel development and germination. Aleurone layers are rich in proteins and on a dry weight basis, contain at least 16.7% protein (Table 1).

Protein content of the starchy endosperm is lower than that of the aleurone tissues. Protein levels of six to nine percent are representative of endosperm tissue. In

Table 1. Protein composition of the various fractions of the rye grain.

Kernel fraction	g/100g*
Whole rye grain	9.9 ⁺
Bran	14.2
Middlings	14.7
Low grade (feed flour)	8.9

after Bushuk, p. 88; * 15% moisture basis;
N x 5.7.

rye, unlike wheat, the concentration of protein decreases toward the centre of the kernel.

2.2 Protein fractions

2.2.1 Cytoplasmic or metabolically active proteins

These proteins are soluble in water and dilute salt solutions. They are enzymes and associated proteins and are located in the cytoplasm. In wheat they are associated with starch granules and are thought to be important in the determination of grain hardness (Barlow et al., 1973; Simmonds et al., 1973).

2.2.2 Storage proteins

Generally, this group are insoluble in water and dilute salt solutions. Osborne (1907) differentiated

storage proteins into prolamins (soluble in 70% ethanol) and glutenins (soluble in dilute acids or alkalis).

Chen and Bushuk (1970) studied the distribution of the various solubility fractions of the proteins of rye, wheat (durum and hard red spring) and triticale. Rye protein was higher in the water soluble and the salt soluble proteins and lower in alcohol and acetic acid solubility fractions than was the protein of the wheats and triticale.

2.3 Genetics of protein inheritance

2.3.1 Genomic control of proteins

Worzella (1934) reported that the seed protein quality of hybrid seed wheat was like that of the maternal parent. More recently, Singh and Nanda (1976) found that seed protein content of F_1 hybrid seed in bread wheat was regulated by the genotype of the maternal parent and not by the genotype of the hybrid endosperm or embryo (on a quantitative basis). Possibly this is a result of the effect of the maternal parent on seed size.

Dhaliwal (1977) made F_1 hybrid seed as well as the reciprocal crosses of diploid, tetraploid and hexaploid wheats. In three of the six crosses made, electrophoretic patterns were different from one another but identical to the maternal plant. Bands characteristic of the paternal parent were missing in the F_1 hybrid seeds. However, in three other crosses, bands which were attributable to the paternal parent were present.

Singh and Hadley (1972) reported that the seed protein of soybeans, Glycine max L., was determined by the maternal genotype. Bingham (1961) reported that milling quality in wheat was determined by the genotype of the endosperm and not that of the maternal parent.

Yong and Unrau (1966) studied the effects of alien genome combinations on the amino acid composition of cereal protein fractions. They reported on rye (Secale cereale RR), durum wheat (Triticum durum - AABB), triticale (AABBRR), tritipyron (AABBEE) and bread wheat (Triticum aestivum - AABBDD). Differences in amino acid profiles of protein fractions of triticale exhibited a dominant effect of either the RR or AABB genomes or was intermediate. The EE and DD genomes were not available for analysis, however, differences observed in the amphiploids were ascribed tentatively to these genomes.

2.3.2 Chromosomal locations of genes for protein

Work in this area has only been done with wheat and rye. Because of the polyploid nature of wheat, chromosome additions or substitutions can be performed in this species. By the use of such aneuploids the effects of single chromosomes have been studied.

Morris et al. (1973) investigated the chromosomal location of genes for high protein in the wheat cultivar 'Atlas 66' by means of monosomic analysis. Chromosomes of the homoeologous group five were involved. Substitution of

5A, 5B and 5D of 'Atlas 66' into 'Chinese Spring' background showed 5D to be definitely involved but 5A and 5B were not clear in their effects.

Waines (1973) examined by electrophoresis the fast moving bands of diploid, tetraploid and hexaploid wheats. He was able to assign certain bands to specific chromosomes while others appeared to be more complex.

Chromosomal control of endosperm proteins was studied by Shepherd (1968), using compensating nullisomic-tetrasomic stocks of wheat and disomic addition lines of 'King II' rye to 'Holdfast' wheat. He showed by the technique of zone electrophoresis that 9 of 17 major bands of 'Chinese Spring' could be accounted for by individual chromosomes of group one or group six. The other eight bands appeared to be controlled by duplicate or triplicate factors on more than one chromosome pair. Most additions of rye chromosomes to 'Holdfast' wheat gave a 'Holdfast' wheat pattern, except the addition of chromosome V (1R), which appeared to be similar to the amphiploid. Possibly all of the factors responsible for the slow moving proteins of 'King II' rye are on one arm of one chromosome.

Chromosome II (2R) of rye was substituted into wheat (Jagannath and Bhatia, 1972) and resulted in an increase in protein content when grown at three locations. No changes in the solubility fractions were observed and no protein bands were seen to be added or deleted on electrophoresis, in comparison with the 'Chinese Spring' wheat

control. They interpreted this as being further evidence of homoeology of rye chromosome II (2R) with wheat group two.

Riley and Ewart (1970) studied the effect of substitution of individual rye chromosomes on the amino acid composition of wheat grains. Their interest was mainly to investigate the possibility of improvement of wheat protein quality by alien transfer, since the amino acid spectrum of rye is superior to that of wheat, especially for lysine. They observed that rye chromosome I (5R) increased wheat lysine by 8.7 percent. This suggests that homoeologous group five may be of significance in the determination of lysine content of wheat grain.

2.3.3 Quantitative studies

A very limited number of studies have been conducted with rye in an attempt to determine the mode of inheritance of its protein levels or to define the range of genetic variation available. Kobyljanskii (1969) reported that from the world rye collection of 2500 forms from 39 countries, there was considerable variation for protein content both between and within cultivars. Among the highest cultivars were two Finnish ryes and forms from Poland and Bulgaria. However, the inheritance of protein content in this material was not studied.

With such a lack of information concerning protein inheritance within Secale cereale, Focke (1956) proposed

interspecific crossing between Secale cereale and Secale montanum as a source of genetic material to be used in the improvement of protein content. Subsequent to the study of Kobyljanskii (1969), a similar interspecific crossing proposal was made by Kotvics (1971).

Muller and Sternkopf (1976) have documented a range in crude protein of 11.8 to 19.0 percent in 98 I₃ (S₃) lines of tetraploid rye. A considerable range was observed in amino acid composition as well. They suggested that the results indicate potential for improvement in tetraploid rye.

Only one study has been conducted in an attempt to define the mode of inheritance of protein content in rye, (Plarre and Fischer, 1975). Their analysis indicated that low protein content is inherited by way of accumulation of dominant alleles and vice versa, high protein content is inherited due to the accumulation of recessive alleles.

Stuber et al. (1962) grew P₁, P₂, F₁, F₂, B₁ and B₂ generations in controlled conditions in an effort to measure with as little interference as possible from the environmental factors, the genetic variation for protein content in Triticum aestivum. Frequency distributions obtained indicated that protein content was under polygenic control.

Miezan et al. (1977) studied the possibility of combined selection for high protein, high yielding wheats. Although a strong environmental influence was observed,

some of the high protein genotypes yielded consistently high and had more protein than the control cultivars in all environments. They concluded that the grain protein of wheat could be increased by breeding without the usual associated decrease in grain yield.

The specific environment in which a particular cultivar is grown may exhibit a great influence on its protein level, therefore, a fixed level of protein is probably an impossible breeding goal to achieve. This is true of most characteristics under the control of many genes, each having a small effect on the particular character in question. Johnson et al. (1973) reported the development of lines of wheat which exhibit high protein levels. Some of the lines maintained their protein advantage over several environments.

In oats, Avena sativa L., Ohm and Patterson (1973b) observed protein content of the F_1 generation to be less than mid-parent values. Percent oat protein was maintained from F_1 to F_2 . They reported hybrid vigour for protein yield in the F_1 , but it disappeared in the F_2 . General combining ability was highly significant for percent oat protein which probably explains the maintenance of protein level from the F_1 to the F_2 generation and its high level of heritability. Usually, partial dominance was shown for low protein.

In Avena sterilis L., Ohm and Patterson (1973a) conducted a six-parent diallel cross. Percent protein in

the F_1 and F_2 generations was similar to the mid-parent values. Ten percent of heterosis was observed in the F_1 population but was much reduced in the F_2 . No reciprocal differences were observed.

Frey et al. (1954) found that the mean percent protein of an F_2 population of oats was near that of the low parent, indicating dominance in the direction of low protein. Sraon et al. (1975) by diallel analysis showed that additive gene action determined oat groat protein percent and that partial dominance for low protein operated in Avena sterilis L.

In crosses of Avena sativa x Avena sterilis, Spilde et al. (1974) found that mean percent protein of progenies was intermediate to the parents. Transgressive segregation was observed for both protein and yield in four of the populations studied suggesting the possibility of improvement of both yield and protein simultaneously.

2.3.4 Time course studies

These studies are generally designed to measure the effects of decreasing water content and the decline in RNA content toward maturity. Brunori et al. (1977) showed that the increased protein content of 'Atlas 66' wheat was due to an extended period of protein synthesis rather than to higher rates of protein accumulation. The cultivar 'Atlas 66' accumulated protein up to the time at which the water content dropped below 30 percent. In contrast, the

cultivar 'Inerio' did not accumulate protein after the water content had fallen to about 50 percent. RNA was not related to the rate of protein accumulation in early seed development, although both cultivars ceased to accumulate protein when RNA content began to decline rapidly.

In legumes where RNA does not decline, the protein continues to accumulate almost to the time at which the seeds are dry (Walbot, 1973). Therefore, it appears that water is not a critical factor in the accumulation of protein but the maintenance of RNA levels is essential. Johnson et al. (1968) have related increased amino acid translocation ability of some cultivars to their higher protein content.

2.4 Correlations

2.4.1 Protein versus yield

The results of many studies have revealed strong negative correlations between protein content and yield. For example, Sraon et al. (1975) observed protein content in oats to be inversely related to yield. However, other researchers have reported weaker correlations. In oats, Ohm and Patterson (1973a,b) reported that the correlations between protein and yield tended to be negative but not significant statistically. They also found that protein percentage versus protein yield tended to be negative but not significant. In wheat, (Johnson et al., 1973) the correlations for yield versus protein in the International Nursery were -0.43 to 0.00, however, correlations for yield versus

protein for varieties not known to be genetically different were -0.61 to +0.65. Moreover, little variation in protein could be accounted for by differences in grain yield even when there was a large negative correlation. Stuber et al. (1962) reported phenotype correlations between protein and yield to be significant but too low to be of any predictive value.

Pandey and Gritton (1976) reported that in peas, (Pisum sativum L.) the correlation between protein and yield was small and inconsistent, therefore, one trait appeared to be independent of the other. Early generation selection was effective and they concluded that progress would depend on the selection intensity and on the choice of parents.

2.4.2 Protein versus lysine

Johnson et al. (1973) found protein to be negatively correlated with lysine content (-0.61 to -0.82) for eight of nine varieties studied. Since approximately one-half of wheat protein is prolamin, they suggested that the genetic advance of lysine content in wheat would involve the reduction of the lysine poor prolamin fraction as was the case with the opaque-2 mutant of maize. Variation for lysine in the world collection of wheat was found to be small (Johnson et al., 1973). However, certain crosses appear to yield transgressive segregates for lysine content and thus there is an opportunity to select for higher lysine levels.