ASPECTS OF PROTEIN CONTENT IN SPRING RYE

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James Grant McLeod

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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

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 ± .10 and 0.55 ± .09 in 1976 and 1977, respectively. Regression of the F_2 generation on the mid-parent values was 0.51 ± .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 triticale 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 I_3 (S_3) 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 P_1, P_2, F_1, F_2, B_1 and B_2 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 groat 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 groat 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.

Kubiczek and Rakowska (1974) reported that low protein genotypes had higher lysine, sulphur amino acids, tryptophan and other amino acids (as a percent of the protein) than high protein types. The absolute amino acid content (as a percent of dry weight) was lower. The amount of useful protein (total protein percent x chemical score), however, increased as protein content increased.

2.5 Other factors

2.5.1 Effect of B-chromosomes

John and Jones (1970) reported on the heterogeneity of soluble proteins as affected by B-chromosomes in rye. Electrophoretically, small differences were observed as the B-chromosomes number was varied. As the number of B-chromosomes was increased the cell size also increased.

Frost (1963a) found that as the number of accessory or B-chromosomes was increased the protein content of the grain also increased. In one case the B-chromosomes were associated with decreased one-thousand kernel weight, while in two other cases the one-thousand kernel weight was increased. In the plants with increased numbers of accessory chromosomes as well as increased protein content, Frost suggested that in some way the accessories stimulated protein production since kernel size was increased. However, one must always keep in mind other effects attributable to B-chromosomes such as fertility and number of kernels per ear. In any case, the presence or absence of

accessory chromosomes should be considered when attempting to study the genetics of protein content in rye.

2.5.2 Seed size and fertility

In material without accessory chromosomes, Frost (1963a) found protein content and kernel size to be negatively correlated. Frost and Ellerstrom (1965) chose tetraploid rye to evaluate the effects of seed size and fertility without the interference of accessory chromosomes. As fertility decreased, protein content increased. Generally, the seed weight and protein content have been found to be negatively correlated in the cereals. Therefore, one would expect tetraploid rye which has a higher one-thousand kernel weight to be lower in protein than the diploid ryes (Müntzing, 1951). Tetraploid rye in fact has higher protein content than diploid rye in all parts of the kernel, i.e. in both aleurone and endosperm.

Frost (1963b) reported on pollen fertility, kernel weight and protein content in 13 inbred lines of rye which were derived from the cultivar Steel. Large differences existed between the lines for fertility (1 to 92%). On the average, fertility in the inbred lines was lower than in the cultivar Steel, however, all lines could be maintained. The mean one-thousand kernel weight was 16.48g with wide variation, moreover, the protein was comparable to the control indicating a more complex situation than is generally thought to exist. The effect of the environment could be

demonstrated as the control cultivar Steel contained 19 to 22 percent protein, whereas, the commercial cultivar Steel contained only 9 to 12 percent protein.

In wheat, Johnson et al. (1973) found a range of 6 to 22 percent in protein content with a mean of 13.0 percent when they screened 12,613 common and 3,400 durum wheats. Seed size profoundly affects the protein percentage by weight. It was found that the correlation of the protein content of F_2 progeny bulks in the F_3 and F_4 generations was only 0.34 but statistically significant. In F_2 bulk populations which had undergone selection for seed plumpness, the F_3 and F_4 correlation was 0.43 and highly significant. Youngs and Peterson (1973) reported higher protein in Avena sterilis than in Avena sativa. However, the one-thousand kernel weight was lower by 50 percent and the percentage of groats was lower (50 percent versus 75 percent) in A. sterilis.

2.5.3 Nitrogen fertilization

No specific reports are available on the effect of nitrogen fertilizers on the protein content of rye grain. In wheat, McNeal et al. (1972) observed elevated protein levels when nitrogen fertilizer was applied. It has also been documented in wheat (Williams, 1966) and barley (Munck, 1964) that where nitrogen is limiting, an inverse relationship between grain yield and protein content exists.

2.5.4 Qualitative factors

Singh and Axtell (1973) screened 9,000 lines of Sorghum bicolor (L.) Moench. They found two floury endosperm mutants (hl) which were high in lysine at relatively high seed protein levels. Feed efficiency ratios (units of feed per unit of gain) were 3.0 for (hl) mutant sorghum and 6.8 for normal (Hl) endosperm sorghum, compared to 3.4 for opaque-2 maize and 7.4 for normal endosperm maize in feeding trials with weanling rats.

Mertz et al. (1964) reported the discovery of an endosperm mutant in maize (opaque-2). Two genes are now known which affect the protein value of maize. The opaque-2 gene increases lysine and tryptophan while the floury-2 increases lysine and methionine. However, certain deleterious factors are associated with the opaque-2 gene. These include softer endosperm which is subject to cracking, lack of seedling vigour and decreased kernel density. Paez (1973) studied the recessive allele at the sugary-2 locus in regards to its effect on the opaque-2 locus in maize, in terms of kernel properties. He found that lysine per unit of protein was higher than in opaque-2 and that kernel density was similar to that of normal endosperm maize. However, other deleterious effects on the kernel type were noted, viz: kernel shrivelling.

The opaque-2 and floury-2 mutants in maize have been shown to block zein synthesis (Lee et al., 1976). The blockage is not complete, however, as some zein fraction is

still produced in these mutants. Gelelectrophoretic analysis of opaque-2 and normal maize revealed that the zein fractions were qualitatively identical except for three missing bands.

Nelson and Burr (1973) have suggested that the only real possibility to improve the quality of protein of cereal endosperms is through the use of such regulatory genes as the opaque-2 and floury-2 which suppress the low lysine storage protein fraction, viz: zein. These genes would only be useful where there is a substantial prolamin or zein fraction present in the endosperm. The prolamin fraction of oats is small, therefore, the possibilities of reducing it still more are limited. Although in wheat a relatively large prolamin fraction exists, the chances of getting a homologous mutant into all three genomes is remote. Rye, on the other hand, is a diploid with a large prolamin fraction. The chances of identifying a regulatory gene which would suppress the synthesis of prolamins in rye would seem to be better than for either wheat or oats, if indeed such a mutant exists.

In barley, a high lysine mutant designated as hyproly was discovered by Hagberg and Karlsson (1969). Subsequent research revealed that the line was inferior in yield and other agronomic characters and that it would be useful only as parent material. No reports of high lysine mutants in oats, wheat or rye are in the literature to date.

Whitehouse (1973) compiled data indicating that improvement in lysine content as a percentage of protein was achieved by the shifting of storage protein from the alcohol soluble fraction to the glutenin, albumin and globulin fractions. Relatively little work has been done in the cereals on protein quality, i.e. amino acid balance, however, the results from nutritional studies document the value of proper balance for both animal and human nutrition (Whitehouse, 1970).

2.5.5 Induced mutants

Only recently has the possibility of improvement of plant protein by nuclear techniques, been actively studied. Major problems exist because of the low probability of detecting suitable mutants (Sigurbjornsson, 1970) and subsequently transferring them to an acceptable agronomic background. Hassan Kahn (1973) reported on the progress and prospects to increase the protein in wheat by mutation breeding. Some lines with increased protein and normal yield levels have been obtained.

2.5.6 DNA, cell size and protein content

Nuclear 4C DNA amounts have been shown to be greater in rye (Bennett et al., 1977) than in barley (Bennett and Smith, 1971). They observed little variation within 2x forms of barley and amounts of DNA in polyploid species were just simple multiples of the 2x forms. In rye, however,

considerable variation, both inter and intraspecific was observed. Rye has about 33 percent more DNA per genome than wheat or oats which are polyploid (Bennett and Smith, 1976).

Ycas et al. (1965) reported that cell volume increased with ploidy and that cell volume and cell mass vary directly as the amount of DNA per nucleus. The amount of a specific protein produced is approximately proportional to the number of structural cistrons in the genome. There is a lack of literature concerning these nuclear aspects and any relationship they may have to seed protein content.

2.6 General statement

Protein content in cereals appears to be under polygenic control. The environment in which a cultivar is grown exerts a strong influence on the level of protein observed. A number of studies of the inheritance of protein content in the cereals have revealed partial to complete dominance of low protein. Several reports in the literature have suggested that protein content and yield can be improved simultaneously in certain cereals. When comparing alternate protein sources, adjustments of protein and yield should be made. A considerable range in protein content has been documented for rye, however, the potential use of this variability in increasing the general protein levels and improvement of protein content of commercial ryes has not been adequately investigated.

MATERIALS AND METHODS

3.1 Protein content of inbred lines of spring rye

In the spring of 1975, 465 inbred lines of spring rye were sown in a field nursery consisting of single row plots, two metres in length and spaced at a width of 30 cm. The seeding rate used was 30 seeds per row. Every tenth row was seeded to the check cultivar Gazelle. The nursery included 345 inbred lines (inbred from four to six generations) which were developed at the University of Manitoba, 80 long term inbreds obtained from D.D. Morey of the Coastal Experiment Station at Tifton Georgia, and 40 S_3 inbred lines obtained from H.H. Geiger (FDR).

To maintain inbred seed, all lines were selfed by placing glassine bags over the spikes prior to anther extrusion and dehiscence. At maturity, yields were determined for each line and a sample of remnant seed was used to determine kernel weight and nitrogen content by Kjeldahl analysis. Protein was reported at zero percent moisture basis.

Means and variances for seed weight and protein content were calculated for Gazelle, the inbreds as a group, and for groups of inbreds derived from various sources. Correlations between protein content and seed weight were

also calculated.

Thirty inbred lines were selected from the inbred nursery grown in 1975 to be studied in further detail. They represented a wide range of protein and seed weight. In 1976 and 1977, the selected inbred lines were grown at Winnipeg in a three-replicate single-row test arranged as a randomized block design. Row length and spacing were the same as those used in the 1975 nursery. The seeding rate used was 30 seeds per metre of row length.

Inbreeding generations of the lines ranged from S_3 to S_x (where x is greater than 10). The 30 inbred lines were characterized as to the original source, the 1975 protein percentage and 200-kernel weight, and the level of inbreeding as shown in Table 2. Data collected on the inbred lines included yield, protein percentage (N x 6.25) and 200-kernel weight.

An analysis of variance was conducted using a fixed model for randomized block design and inbred line means were compared for the characters measured. Phenotypic correlations also were calculated.

3.2 Protein content of F_1 hybrids of spring rye

Since large differences in protein content were observed in the inbred lines, the genetic basis for such variability was investigated. Thirty inbred lines were selected on the basis of protein content, from the field nursery grown at Winnipeg during the summer of 1975. No

Table 2. Characteristics of the inbred lines chosen for study in 1976 and 1977.

Inbred line	Source	Protein %	200-kwt.(g)
2019 S_7	Apizaco	24.1 H*	3.72
2039 S_6	Petka	17.7 L	3.73
2066 S_7	O.J. Rye	24.6 H	3.33
2076 S_7	PI 173599	16.0 L	4.09
2121 S_5	Self-Fertile Spring	16.7 L	3.43
2125 S_5	" " "	23.7 H	3.56
2136 S_7	" " "	17.5 L	3.75
2141 S_6	Petka	23.0 H	3.19
2178 S_7	Prolific	23.4 H	4.80
2207 S_7	Self-Fertile Prolific	25.9 H	4.30
2214 S_6	" " "	16.8 L	3.28
2219 S_7	O.J. Rye	24.4 H	4.01
2237 S_7	Prolific	23.8 H	3.99
2241 S_7	Self-Fertile Prolific	25.8 H	3.41
2247 S_6	PI 323387	23.7 H	5.38
2266 S_7	Self-Fertile Prolific	24.8 H	3.38
2296 S_7	" " "	16.4 L	3.48
2308 S_6	" " Spring	25.9 H	3.19
2324 S_5	Korn	17.9 L	5.11
2380 S_6	PI 170984	17.8 L	4.44
2401 S_7	PI 323387	23.2 H	4.40
2406 S_5	"	23.2 H	5.20
2513 S_x†	Kenya	28.7 H	3.60
2547 S_x	Gator	26.4 H	4.00
2549 S_x	"	23.8 H	4.00
2575 S_3	Kings II x Petkuser	23.7 H	4.40
2579 S_3	" "	24.5 H	3.80
2581 S_3	" "	23.9 H	4.76
2587 S_3	" "	16.2 L	3.79
2590 S_3	" "	15.4 L	5.11

* H = high protein, L = low protein. † x is greater than 10.

B-chromosomes were present in the lines studied. The lines were of diverse origin and represented a considerable range of protein levels (Table 2, section 3.1). Using a number of these selected lines, 30 F_1 hybrid populations were produced in the greenhouse during the winter of 1975 to 1976. Hybrids were produced on the basis of synchrony of flowering and on their protein level so that a sample of crosses combining high-protein parents, low-protein parents and high- and low-protein parents was obtained. Emasculation was by way of the sissor technique while for pollination, bulk pollen was collected from several plants within the designated inbred parental line. Sufficient pollinations were carried out to obtain at least 600 hybrid seeds. The 30 F_1 hybrids produced were derived from 13 high x high, 10 low x low and seven low x high parental combinations.

During the summers of 1976 and 1977, the F_1 hybrid generation together with the parents was grown at Winnipeg in a three-replicate, randomized block design. Plots consisted of single rows, two metres in length and spaced at a width of 30 cm. The seeding rate was 50 seeds per metre of row. The F_2 generation was grown in 1977 at Winnipeg in an experimental design similar to that used for the F_1 populations.

The data collected included yield in grams per plot, percentage crude protein (N x 6.25) and 200-kernel weight in grams. The analysis of variance was conducted using a fixed model for a randomized block design. Regres-

sions of F_1 hybrid protein percentage on mid-parent values were calculated to determine the predictive value of mid-parent protein percentage for F_1 and F_2 progenies. Comparisons also were made between the groups of hybrids, i.e. high x high, low x low and high x low groups. Rank correlation coefficients were computed between grain yield, protein yield (product of grain yield and protein content), protein percentage and 200-kernel weight.

3.3 Six-parent diallel cross

Six inbred lines of spring rye, Secale cereale L., of diverse origin were selected for this study (Tables 3a, 3b). Each represented five to seven generations of inbreeding (S_5 to S_7) and theoretically were considered to be homozygous at more than 95 percent of their loci. Their range in crude protein content was approximately 10 percent.

In 1976 the inbred lines were crossed in all combinations to derive a set of reciprocal F_1 hybrids. The emasculations were carried out using the sissors technique. For any given pollination, bulk pollen was collected from several plants within the designated parental inbred line. Sufficient pollinations were carried out to obtain 400 to 500 F_1 seeds from each cross.

The F_2 generation was derived by selfing 20 to 30 reciprocal F_1 plants by means of placing glassine bags over the spikes prior to the time of anther extrusion and dehiscence. About 500 F_2 seeds were produced from each selfed

Table 3a. Characterization of the spring rye inbred lines used in the six-parent diallel cross (1975 data).

Inbred	Source	Inbred generation	Height (cm)	Days to heading	200-kwt.	Crude protein (%)
A	Apizaco	S_7	97	48	3.72	24.1
B	O.J. Rye	S_7	105	54	3.33	24.6
C	PI 173599	S_7	95	50	4.09	16.0
D	Self-Fertile Spring	S_5	91	54	3.43	16.7
E	Self-Fertile Prolific	S_7	83	57	4.30	25.9
F	PI 170984	S_6	103	49	4.44	17.8

Table 3b. Characterization of the spring rye inbred lines used in the six-parent diallel cross (1976 and 1977 data).

Inbred	Source	Inbred generation	Height (cm) mean	Height (cm) S.E.	Days to Head mean	Days to Head S.E.	200-kwt. (g) mean	200-kwt. (g) S.E.	Crude protein (%) mean	Crude protein (%) S.E.
A	Apizaco	S_8	90	0.9	56	1.2	3.59	0.25	23.0	0.7
B	O.J. Rye	S_8	92	2.6	61	0.0	3.42	0.05	23.9	0.7
C	PI 173599	S_8	83	2.1	56	0.9	4.18	0.04	13.4	0.1
D	Self-Fertile Spring	S_6	86	1.9	59	0.0	3.44	0.14	16.0	0.1
E	Self-Fertile Prolific	S_8	88	5.8	57	1.2	4.39	0.31	22.0	1.0
F	Pl 170984	S_7	92	1.2	57	0.9	4.43	0.08	18.7	0.3
A	Apizaco	S_9	101	1.3	58	0.4	4.21	0.08	22.5	0.1
B	O.J. Rye	S_9	108	2.8	63	0.2	4.10	0.06	23.3	0.2
C	PI 173599	S_9	97	1.8	62	0.3	4.18	0.13	12.3	0.2
D	Self-Fertile Spring	S_7	107	1.9	62	0.1	3.96	0.08	14.1	0.2
E	Self-Fertile Prolific	S_9	95	1.4	63	0.2	5.01	0.07	22.5	0.2
F	Pl 170984	S_8	100	1.3	62	0.1	5.05	0.06	18.8	0.2

F_1 population. Twenty to 30 plants of the F_2 generation were selfed at random to derive F_3 lines so that 20 to 30 F_3 lines were synthesized for each reciprocal cross. No selection was done on F_2 plants.

During the summer of 1977, the F_1 and F_2 generations were grown at two locations (Winnipeg and Glenlea). Two replicates were sown at each location in a randomized block design. Plots consisted of single rows, 2 metres in length and spaced at a width of 30 cm. The seeding rate was 50 seeds per metre of row. The F_3 generation, because of lack of seed, was sown in one replicate at Winnipeg only, the lines from reciprocal crosses being kept separate in two blocks. Crosses were randomized and progenies were sown as a group of lines.

Data collected included yield in grams per plot, percentage crude protein determined by Kjeldahl analysis and 200-kernel weight. Data were analyzed by the use of Griffing's (1956) method 3, model I to obtain estimates of general combining ability (GCA) and specific combining ability (SCA) for the F_1 and F_2 generations. Method 4, model I of Griffing (1956) was employed in the analysis of the F_3 generation where the reciprocals were considered as the second replicate.

Inbreeding depression was calculated from F_1 and F_2 data. The formula used was:

$$\frac{\bar{F}_1 - \bar{F}_2}{\bar{F}_1} \times 100\%.$$

RESULTS AND DISCUSSION

4.1 Protein content of inbred lines of spring rye

The check cultivar Gazelle, spaced at 10-row intervals throughout the nursery, had the smallest variance (0.32) when compared to the various groups of inbred lines (Table 4). The variance of values of percentage protein obtained for Gazelle may be taken as a measure of the environmental variance for the research area. However, its heterozygosity and heterogeneity may confer on it a substantially greater buffering capacity against environmental differences than may have been the case had an inbred line been used. The environmental variance as measured by Gazelle, therefore, may be biased downwards compared to the environmental variance of the inbreds. The mean value for protein percentage was also lower for Gazelle (18.6) than for any group of inbred lines.

Variances of inbreds derived from the cultivar Prolific tended to be low (0.70 to 2.83), while the variation of inbred lines derived from Self-Fertile Spring were much higher (9.24 to 12.19), (Table 4). Possibly the small number of plants giving rise to the inbred lines of Prolific limited the genetic base. Secondly, there may have been less inherent variability in the cultivar Prolific than in

Table 4. Means, ranges and variances of protein content (%) of Gazelle check and inbred lines in the single-row nursery, 1975.

Source	Number of lines	Mean	Range	S^2
Gazelle (check)	68	18.6	17.5-20.2	0.32
Inbreds	465	21.5	15.4-30.4	6.35**
U. of M. Inbreds	345	21.9	16.4-30.4	6.23**
S.F.P. (all)+	93	20.9	16.4-26.3	4.32**
" S_4	14	19.4	17.8-23.4	1.81**
" S_5	20	20.2	16.8-24.7	5.15**
" S_6	58	21.4	16.4-26.3	3.46**
Prolific	22	21.8	18.3-24.2	2.83**
" S_4	8	23.2	21.4-24.2	0.70*
" S_6	14	20.9	18.3-23.8	2.30**
S.F.S. (all)+	58	22.1	16.7-28.6	12.04**
" S_4	11	20.1	16.7-26.9	9.24**
" S_5	18	23.9	17.4-27.6	12.19**
" S_6	25	21.6	17.5-28.6	10.84**
Korn	18	22.3	17.9-25.3	3.44**
" S_5	5	22.5	19.8-24.0	2.50**
" S_6	12	22.7	20.7-25.3	2.50**
Others	152	22.5	17.7-30.4	1.65**
Georgia (L.T.)+	80	21.2	17.4-26.6	4.11**
Germany S_3	40	19.1	15.4-24.5	4.39**

+ S.F.P. = self-fertile prolific, S.F.S. = self-fertile spring, L.T. = long term inbred.

*,** Significantly greater variance than Gazelle at 5% and 1% levels of probability, respectively.

Self-Fertile Spring. Inbred lines of Self-Fertile Prolific were derived from Prolific and showed a variance of 4.32 which is similar to that of inbreds derived from the cultivar Korn (3.44), Geiger's S_3 inbreds (4.39) and the long term inbreds from Georgia (4.11). The variance of protein content of inbreds from Self-Fertile Prolific was higher than the variance of inbreds derived from the cultivar Prolific, (4.32 vs 2.83). The sample size of Self-Fertile Prolific inbred lines was over four times as large as that of Prolific inbreds. On the other hand, inbreds of Self-Fertile Spring showed a variance much larger than that of inbred lines of Self-Fertile Prolific (12.04 vs 4.32), although the sample size was smaller.

It is also of interest that S_3 lines obtained from Germany are as differentiated for protein content as are later generation inbreds, i.e. long term inbreds from Georgia. Because variation tends to remain constant in the inbreds with continued inbreeding beyond the S_3 generation, selection for protein content could be initiated at that time. Possibly selection could be practised as early as the S_1 or S_2 generation.

One may hypothesize that protein content would be controlled by fewer genes than yielding ability. Therefore, it may be possible to screen for protein content on the basis of S_1 plant performance, thereby circumventing other confounding problems such as inbreeding depression and its concomitant effect on protein content.

The variance of seed weight of the groups of inbred lines was greater than Gazelle with the exception of the group of inbreds derived from Prolific (Table 5). The range of seed weights of inbred groups did not overlap with the range of Gazelle seed weights with the exception of some of the S_3 inbreds obtained from Germany. The mean kernel weight of the inbreds as a group was approximately 50 percent of that of Gazelle. The mean kernel weight of inbreds which were obtained from Germany was approximately 68 percent of Gazelle.

Phenotypic correlation coefficients for percentage protein versus 200-kernel weight are presented in Table 6. The correlation was significant for the check cultivar Gazelle and also for major groups of inbreds (U. of M., Georgia and Germany). However, subgroups of the U. of M. inbreds showed a number to be non-significant, especially in the Prolific and Self-Fertile Prolific groups. In these two subgroups only one 'r' value was significant (S_4 Prolifics) although it should be noted that there was a very limited number of lines in this group. Only one correlation was positive (and non-significant) while all others were negative. There was a definite negative trend in correlations between seed weight and protein content as should be expected.

Yields of the inbred lines were not analysed in 1975. Their yields were low and probably not reliable due to the low seeding rate.

Table 5. Means, ranges and variances of 200-kernel weight (g) of Gazelle check and inbred lines in the single-row nursery, 1975.

Source	Number of lines	Mean	Range	S^2
Gazelle (check)	68	6.80	6.00-7.60	0.23
Inbreds	465	3.68	1.60-6.40	0.77**
U. of M. Inbreds	345	3.56	1.60-6.00	0.71**
S.F.P. (all)+	93	3.44	2.40-5.20	0.31
" S_4	14	3.20	2.80-4.00	0.13
" S_5	20	3.40	2.00-5.20	0.52**
" S_6	58	3.48	2.00-4.80	0.27
Prolific	22	3.76	2.40-4.80	0.27
" S_4	8	3.64	3.20-4.00	0.06
" S_6	14	3.80	2.40-4.80	0.41
S.F.S. (all)+	58	3.28	2.00-5.20	0.36*
" S_4	11	3.76	2.80-5.20	0.41
" S_5	18	3.16	2.00-4.40	0.41
" S_6	25	3.20	2.40-4.40	0.31
Korn	18	3.56	2.80-5.60	0.64**
" S_5	5	3.52	3.20-4.80	0.52
" S_6	12	3.40	2.80-4.80	0.36
Others	152	3.68	1.60-6.00	1.00**
Georgia (L.T.)+	80	3.72	2.00-6.00	0.77**
Germany S_3	40	4.68	3.20-6.40	0.52**

+ S.F.P. = self-fertile prolific, S.F.S. = self-fertile spring, L.T. = long term inbred.

*,** Significantly greater variance than Gazelle at 5% and 1% levels of probability, respectively.

Table 6. Phenotypic correlations between protein content (%) and 200-kernel weight for Gazelle check and inbred lines in the single-row nursery, 1975.

Source	Number of lines	Correlation
Gazelle (check)	68	-0.20*
Inbreds	465	-0.36**
U. of M. Inbreds	345	-0.26**
S.F.P. (all)+	93	-0.03
" S_4	14	-0.22
" S_5	20	-0.09
" S_6	58	-0.11
Prolific	22	-0.03
" S_4	8	-0.48*
" S_6	14	0.14
S.F.S. (all)+	58	-0.54**
" S_4	11	-0.20
" S_5	18	-0.44*
" S_6	25	-0.66**
Korn	18	-0.71**
" S_5	5	-0.95**
" S_6	12	-0.34
Others	152	-0.33**
Georgia (L.T.)+	80	-0.35**
Germany S_3	40	-0.25*

+ S.F.P. = self-fertile prolific, S.F.S. = self-fertile spring, L.T. = long term inbred.

*,** Correlation significant at 5% and 1% levels of probability, respectively.

The range of values for protein content in rye which were documented by Muller and Sternkopf (1976) was 11.8 to 19.0 percent. They reported on I_3 (S_3) lines of tetraploid rye, whereas the present study included only diploid rye lines. The ranges and means of protein percentage of inbred lines in the present study were generally higher than those of the Muller and Sternkopf study. However, this could be the result of differences in nitrogen fertility levels and genetic factors as well as level of inbreeding. The I_3 lines of tetraploid rye represented approximately one generation of inbreeding in a diploid.

Analysis of variance of yield, protein percentage and 200-kernel weight are presented in Table 7 for the inbreds grown in 1976 to 1977. The analysis indicated highly significant differences between inbreds for all three characters. Pooling of the data for the two years revealed a highly significant year effect, therefore, the environment affected the performance of the inbred strains each year. No interaction was detected for yeild, while a strong interaction (year x inbred) was observed for protein percentage and 200-kernel weight. Further partitioning of the variances revealed significant differences between inbreds and for check versus inbreds comparisons for both 1976 and 1977 for the three characters analysed.

The 30 selected inbreds could be classified by percentage protein into two distinct groups utilizing the protein determinations from the single-row nursery in 1975

Table 7. Analyses of variance of protein content (%), yield (g/plot) and 200-kernel weight (g) of 30 inbreds and Gazelle, grown in 1976 and 1977.

Source	df	Mean square		
		Protein (%)	Yield (g/plot)	200-kwt.(g)
			1976	
Blocks	2	0.10	577.57	0.05
Entries	30	43.03**	19059.70**	2.18**
Inbreds	29	42.90**	8645.90**	1.48**
Check vs inbreds	1	46.76**	321059.82**	22.41**
Error	60	0.99	559.43	0.07
			1977	
Blocks	2	0.33	511.78	0.05
Entries	30	44.87**	16478.44**	2.06**
Inbreds	29	44.98**	9418.74**	1.45**
Check vs inbreds	1	41.60**	221209.63**	19.77**
Error	60	0.36	477.97	0.05

(Table 2). The high protein group of 20 lines had a range of 23.0 to 28.7 percent protein, while the low group of 10 lines had a range of 15.4 to 17.9 percent. On the basis of replicated test data obtained in 1976 and 1977, only three of the lines appear to be incorrectly classified by the single-row data obtained in 1975 (Tables 8 and 9). Lines 2125 and 2575 were classfied as high protein lines in 1975 and were in the low group in 1976 and 1977. The line 2324 was classified in the low group in 1975 and was in the high group in 1976 and 1977. The wrong classifications could have been the result of a real genotype x environment interaction.

Protein levels in 1976 and 1977 were on average lower than in 1975. Somewhat better moisture conditions which prevailed in 1976 and 1977 could have contributed to this difference.

Phenotypic correlations of protein percentage, yield in grams per plot and 200-kernel weight are presented in Table 10. Protein percentage versus yield was negative in all three years and significant at the 5 percent level in 1976 and 1977. Protein percentage versus kernel weight was negative, but non-significant in all three years. Kernel weight was strongly correlated with yield in 1975 and 1976, but not in 1977.

The 10 highest ranked lines for yield in 1976 and 1977 are presented in Table 11. The lines 2308 and 2590 were ranked first and second, respectively, in both years.

Table 8. Means of protein percentage, 200-kernel weight and yield of 30 inbred lines at Winnipeg in 1976.

Line	Protein (%)	200-kwt.(g)	Yield (g/plot)
2019	23.0	3.59	34
2039	17.8	4.25	115
2066	23.9	3.42	39
2076	13.4	4.18	78
2121	16.0	3.44	15
2125	15.4	3.61	25
2136	16.9	3.92	101
2141	22.5	3.24	38
2178	25.0	3.23	5
2207	22.0	4.39	91
2214	14.3	3.69	124
2219	21.4	4.64	56
2237	22.3	3.87	51
2241	22.8	3.74	81
2247	23.4	4.92	6
2266	24.6	3.56	19
2296	15.6	3.99	103
2308	20.2	5.22	243
2324	23.2	4.34	93
2380	18.7	4.44	88
2401	22.9	3.86	6
2406	21.8	4.82	10
2513	26.4	3.08	22
2547	23.7	2.52	23
2549	20.9	2.23	10
2575	17.0	4.84	24
2579	23.2	4.35	71
2581	25.5	4.36	53
2587	15.7	3.58	65
2590	15.5	4.77	165
Gazelle	16.5	6.72	394
S.E.	0.8	0.22	19

Table 9. Means of protein percentage, 200-kernel weight and yield of 30 inbred lines at Winnipeg in 1977.

Line	Protein (%)	200-kwt. (g)	Yield (g/plot)
2019	23.8	4.37	43
2039	17.3	4.13	147
2066	23.5	4.03	65
2076	12.4	3.93	45
2121	14.1	3.90	28
2125	13.6	3.63	30
2136	16.1	4.00	116
2141	22.2	3.63	56
2178	27.5	3.23	7
2207	22.9	4.63	31
2214	14.4	3.33	140
2219	21.4	4.30	65
2237	21.0	3.83	61
2241	20.2	3.80	114
2247	22.8	5.27	21
2266	24.5	3.77	25
2296	16.5	4.10	150
2308	20.4	4.87	229
2324	19.6	3.93	104
2380	19.2	4.90	80
2401	22.6	4.87	10
2406	19.6	5.83	13
2513	23.8	4.40	22
2547	20.7	3.23	40
2549	21.1	2.33	10
2575	17.5	4.90	36
2579	23.2	4.37	77
2581	22.5	4.50	64
2587	14.4	3.67	76
2590	14.4	4.57	186
Gazelle	16.1	6.77	346
S.E.	0.5	0.18	18

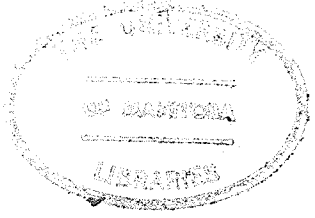
Table 10. Phenotypic correlation coefficients for protein percentage, yield in grams per plot and 200-kernel weight in 1975, 1976 and 1977.

Characters	Years		
	1975	1976	1977
Protein percentage vs yield	-0.34	-0.40*	-0.39*
Protein percentage vs 200-kwt.	-0.20	-0.26	-0.03
Yield vs 200-kernel weight	0.52**	0.70**	-0.07

*,** Correlation coefficient significant at the 5% and 1% levels, respectively.

Table 11. Rank and protein levels of the 10 highest yielding inbred lines in 1976 and 1977.

Line	Protein level	Yield rank	
		1976	1977
2308	high	1	1
2590	low	2	2
2214	"	3	5
2039	"	4	4
2296	"	5	3
2136	"	6	6
2324	"	7	8
2207	high	8	-
2380	low	9	9
2241	high	10	7
2579	"	-	10



Although three high protein lines were ranked among the 10 highest yielding lines in both years, there was an excess of low protein lines (seven). The proportion of high protein lines in the 10 highest yielders may have been even smaller had an equal number of inbreds been included in both the high and low protein classes. Only 10 of the 30 lines in the test were classed as low in protein content.

4.2 Protein content of F_1 hybrids of spring rye

Analysis of variance of the 30 F_1 hybrid populations grown for two years at Winnipeg revealed significant differences among hybrids for protein percentage, yield in grams per plot and 200-kernel weight (Table 12). Significant differences were also detected for the three characters in the F_2 generation. When the F_1 generation data were combined over the two years, yield and 200-kernel weight showed a significant year x hybrid interaction.

Protein percentages significantly greater than Gazelle were detected in 11 of the hybrids in both years (Table 13). In 1976 no hybrid outyielded Gazelle significantly. However, seven of the hybrids were not significantly different from Gazelle in yield and three of these were significantly greater than Gazelle in protein percentage. Only one hybrid was significantly greater than Gazelle in seed weight. In the F_2 generation, nine of the 11 hybrids superior to Gazelle in protein percentage in the F_1 generation were still superior. However, for yield no hybrid was

Table 12. Analyses of variance of 30 F_1 hybrids tested at Winnipeg in 1976 and 1977 and 30 F_2 hybrids tested in 1977.

Source	df	Mean square		
		Protein (%)	Yield (g)	200-kwt.(g)
F_1 1976				
Blocks	2	0.50	4828.2*	0.07
Hybrids	30	14.59**	12412.4**	1.08**
Block x Hybrid	60	0.79	1699.6	0.06
F_1 1977				
Blocks	2	0.20	5605.6	0.36**
Hybrids	30	14.72**	14027.6**	1.97**
Block x Hybrid	60	0.17	2732.6	0.04
F_2 1977				
Blocks	2	0.57	2169.3	0.64**
Hybrids	30	12.53**	12511.7**	0.95**
Block x Hybrid	60	0.21	1103.5	0.07

*,** Mean squares are significant at 5% and 1% probability levels, respectively.

Table 13. Means of protein percentage, yield (g/plot) and 200-kernel weight (g) for F_1 and F_2 hybrid tests.

Hybrid	Protein (%)			Yield (g/plot)			200-kwt. (g)		
	1976 F_1	1977 F_1	1977 F_2	1976 F_1	1977 F_1	1977 F_2	1976 F_1	1977 F_1	1977 F_2
1	16.6	17.0	15.7	370	392	244	5.9	6.0	4.5
2	17.0	17.6	16.0	291	376	227	4.5	4.8	3.8
3	13.8	14.8	15.0	313	352	273	4.8	4.7	4.5
4	15.1	14.8	16.3	296	384	357	5.6	5.7	5.8
5	13.3	13.5	13.9	256	351	248	5.2	5.3	4.3
6	19.9	20.0	19.1	304	343	196	5.8	6.1	4.6
7	14.8	14.5	14.2	345	405	325	5.8	6.2	4.6
8	17.6	19.2	17.4	380	457	373	7.2	8.0	5.4
9	17.9	17.5	19.2	248	338	230	6.6	6.7	5.2
10	16.3	17.1	19.4	223	530	335	5.4	5.5	4.8
11	14.4	14.9	14.1	332	463	260	6.8	7.2	4.5
12	13.1	14.5	14.2	302	432	255	6.3	7.4	4.8
13	18.7	18.9	19.0	385	476	330	5.7	5.9	4.9
14	16.5	16.7	18.4	333	389	287	5.0	4.7	4.6
15	17.8	18.3	18.5	330	427	174	5.6	5.8	4.4
16	15.9	15.7	15.8	354	467	283	5.8	5.9	4.4
17	20.5	19.8	20.5	270	417	198	5.7	5.5	4.4
18	13.2	12.8	14.4	245	348	317	5.3	5.4	4.3
19	19.4	19.0	19.9	257	353	176	5.3	5.4	4.6
20	18.6	18.9	19.7	485	537	306	6.0	6.4	4.6
21	15.3	13.5	16.1	337	551	321	5.5	5.1	4.7
22	17.3	17.1	18.4	284	407	238	5.8	5.9	5.2
23	19.5	19.3	17.5	261	403	237	5.1	5.0	5.1
24	19.4	19.3	19.6	317	465	341	6.3	6.4	4.5
25	18.8	19.4	19.5	276	516	293	6.2	6.3	4.4
26	14.5	16.8	17.0	397	526	435	5.8	5.3	5.1
27	14.8	15.3	16.2	383	421	232	5.9	6.1	4.8
28	13.5	13.1	15.7	460	572	364	5.5	5.6	4.9
29	14.7	14.2	14.9	269	381	258	4.9	4.8	4.0
30	14.9	14.8	15.2	320	477	278	6.3	6.6	5.1
Gazelle	16.0	16.1	16.8	434	466	398	6.5	7.1	6.7
L.S.D.(.05)	1.5	0.9	0.8	67	85	54	0.4	0.3	0.4

significantly greater than Gazelle and only four were not significantly different from Gazelle. All F_2 populations were significantly lower than Gazelle in seed weight. Reduction in yield and seed weight of the F_2 hybrids can probably be attributed to inbreeding depression.

Without exception, inbreeding depression was observed to be positive for yield (Table 14) and in all except one population for 200-kernel weight. Negative inbreeding depression of protein percentage was observed in most hybrids as was expected. However, no trend was apparent in hybrids showing positive inbreeding depression of protein, i.e. hybrids showing inbreeding depression did not always fall in a particular group of crosses.

Mid-parent protein versus F_1 hybrid protein percentages are plotted in Figure 2. When mid-parent protein percentage was low, the F_1 progeny protein percentage was also low. The same trend was observed in both 1976 and 1977, although in 1977 there was somewhat more overlap of the progeny of low protein and high protein parents with the progeny of high and low protein parents. The same trend was apparent when the results of progeny protein tests were plotted against mid-parent values for the F_2 generation (Table 15).

Table 14. Inbreeding depression in percent of protein percentage, yield and 200-kernel weight (from 1977 data).

Hybrid	Protein (%)	Yield (g/plot)	200-kwt. (g)
1	7.5	37.8**	25.0**
2	9.3	39.6**	20.8**
3	- 1.6	22.4*	4.3
4	- 9.9	7.0	1.8
5	- 3.0	29.3**	18.9**
6	4.5	42.9**	24.6**
7	1.9	35.6**	25.8**
8	9.4	18.4*	32.5**
9	- 9.9	32.0**	22.4**
10	-13.6*	36.8**	12.7*
11	5.2	43.8**	23.6**
12	2.1	41.0**	35.1**
13	- 0.5	30.7**	17.0*
14	-10.4*	26.2*	2.1
15	- 0.9	59.3**	24.1**
16	- 0.5	39.4**	25.4**
17	- 3.5	52.5**	20.0**
18	-12.5*	8.9	20.4**
19	- 4.6	50.1**	14.8*
20	- 4.1	43.0**	28.1**
21	-19.5**	41.7**	7.8
22	- 7.4	41.5**	11.9*
23	9.2	41.2**	- 2.0
24	- 1.4	26.7*	29.7**
25	- 0.5	43.2**	30.2**
26	- 1.0	17.3	3.8
27	- 5.9	44.9**	21.3*
28	-20.1**	36.4**	12.5*
29	- 5.1	32.3**	16.7*
30	- 2.9	41.7**	22.7**
Gazelle	- 4.5	14.6	5.6

*,** Inbreeding depression significant at 5% and 1% probability levels, respectively.

Table 15. Regression of progeny protein values on mid-parent protein values for 30 hybrids

Regression	Y-intercept	b	S.E.	r
F_1.MP 1976	5.93	0.52**	.10	.78
F_1.MP 1977	5.94	0.55**	.09	.83
F_2.MP 1977	7.07	0.51**	.08	.84

** b is significantly greater than 0 at 1% probability level.

It is noteworthy that the regression coefficients are similar for the F_1 population in both years and that the F_2.MP regression is similar to that of the F_1.MP. The Y-intercept changes in the F_2.MP regression, probably due to the effects of inbreeding on seed weight and yield and the resulting increase in protein content. These results of this experiment provide support for the use of 1975 data for classification of high and low protein.

The means and ranges of protein percentage for the progeny of the various groups of crosses are presented in Table 16. The range in protein level of the hybrids was approximately four percent in both years with a two percent difference between the H x L, L x L and H x H groups. The protein levels were greater in the H x H group of crosses than in either of the L x L or H x L groups.

Table 16. Means of groups of crosses (protein %, protein yield and grain yield) entered in the 30 hybrid test in 1976 and 1977.

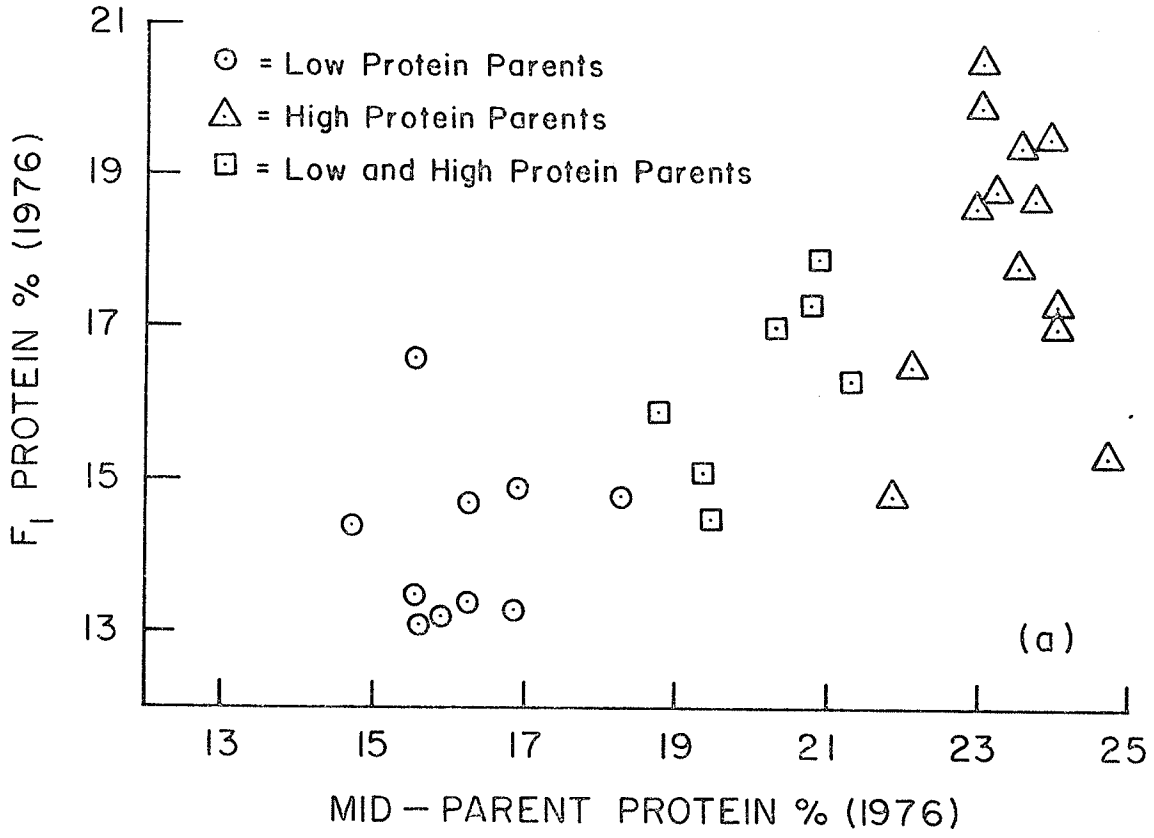
Character	Cross	Entries	F_1 generation 1976	F_1 generation 1977	F_2 generation 1977
Protein %	H x H	13	18.2±.5	18.5±.4	18.5±.5
	L x L	10	14.2±.3	14.5±.4	14.9±.2
	H x L	7	16.4±.4	16.2±.6	17.3±.6
	Gazelle	1	16.0±.3	16.1±.4	16.1±.3
Grain yield (g)	H x H	13	344±18	447±18	282±23
	L x L	10	325±21	419±22	280±16
	H x L	7	290±17	436±31	284±21
	Gazelle	1	434±18	466±20	398±20
Protein yield (g)	H x H	13	60.1±3.9	82.3±3.3	51.7±3.8
	L x L	10	45.2±3.9	60.6±3.3	40.9±2.1
	H x L	7	49.1±2.2	70.0±4.3	49.1±3.7
	Gazelle	1	69.4±2.7	75.0±3.0	70.0±3.1

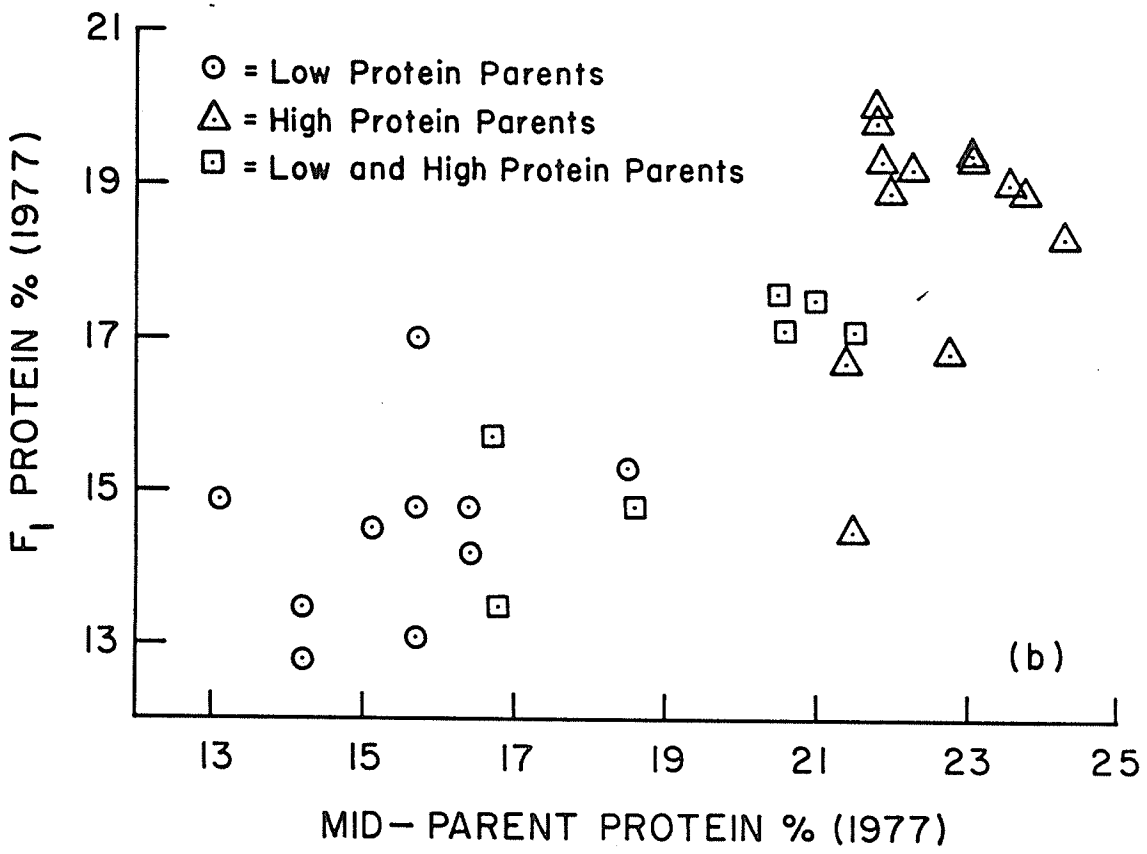
Figure 1. Plots of progeny protein levels versus mid-parent protein levels.

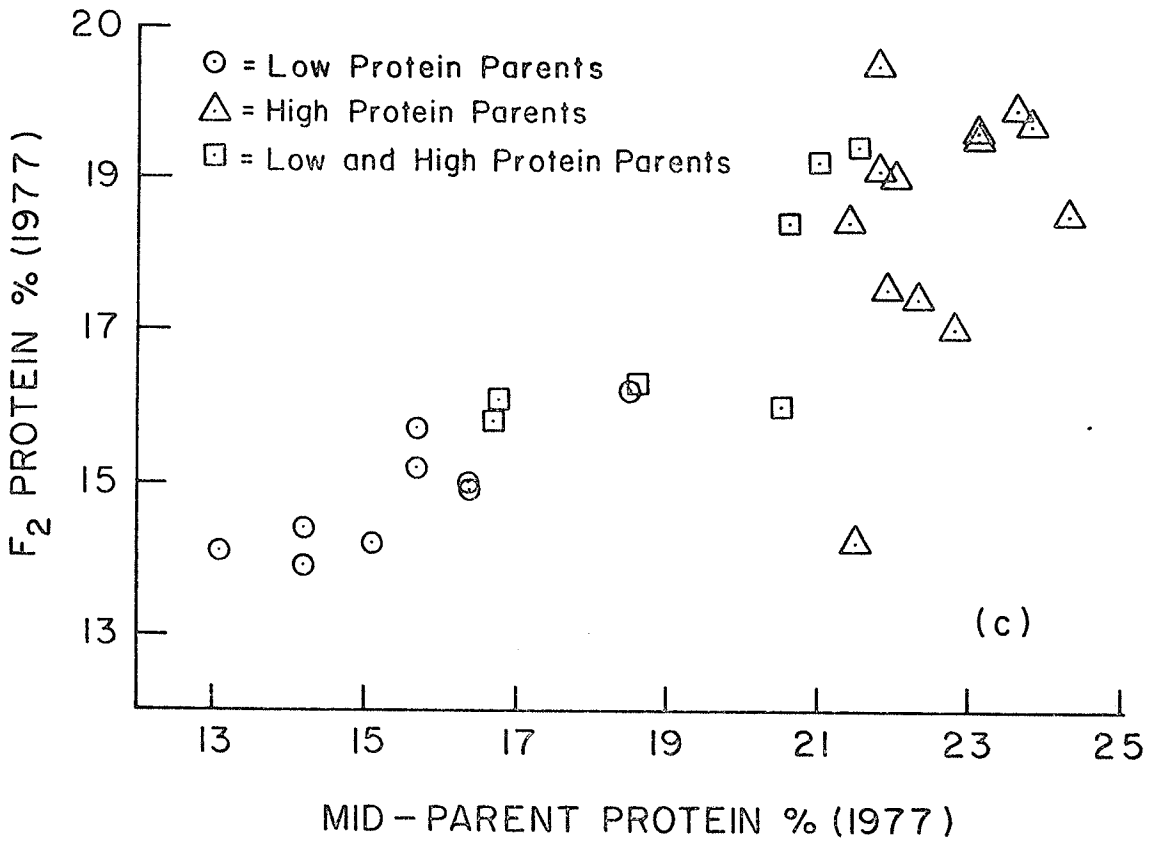
a) F_1 progeny protein percent versus mid-parent protein percent (1976).

b) F_1 progeny protein percent versus mid-parent protein percent (1977).

c) F_2 progeny protein percent versus mid-parent protein percent (1977).


21
19
17
15
13
F1 PROTEIN % (1976)
⊙ = Low Protein Parents
△ = High Protein Parents
⊡ = Low and High Protein Parents
(a)
13
15
17
19
21
23
25
MID – PARENT PROTEIN % (1976)


⊙ = Low Protein Parents
△ = High Protein Parents
⊡ = Low and High Protein Parents
(b)
F_1 PROTEIN % (1977)
MID-PARENT PROTEIN % (1977)
21
19
17
15
13
13
15
17
19
21
23
25


⊙ = Low Protein Parents
△ = High Protein Parents
⊡ = Low and High Protein Parents
(c)
20
19
17
15
13
13
15
17
19
21
23
25
F2 PROTEIN % (1977)
MID-PARENT PROTEIN % (1977)

Grain yield was higher in 1977 than in 1976. The progeny of high protein parents had the highest mean yield as a group in each year. In both years the absolute yield of the cultivar Gazelle was greater than the mean of any group of hybrids. The mean yield of Gazelle was significantly greater than the mean yield of all three groups of hybrids in 1976 but in 1977 Gazelle was only significantly greater than the L x L group. In the F_2 generation, the yield of Gazelle was significantly greater than all three groups of hybrids. There were no significant differences between the three groups in the F_2 generation.

Protein yield was the lowest in the progeny of low protein parents in both the F_1 and F_2 generations. Gazelle gave the highest protein yield in 1976 but was lower than the mean of the high protein group of crosses in 1977.

Protein yield of three hybrids from the H x H group was equal to or greater than Gazelle in 1976, while no hybrid from either of the L x L or H x L (L x H) groups had protein yield equal to Gazelle. In 1977, 10 of the hybrids from the H x H group outyielded Gazelle in protein, while three of the hybrids from each of the L x L and H x L (L x H) groups had protein yield equal to or greater than Gazelle.

Rank correlation coefficients (Table 17) were negative and non-significant for protein percentage versus grain yield, indicating a lack of relationship between these two characters. However, protein percentage and pro-

Table 17. Spearman's rank correlation coefficients of yield, protein %, protein yield and 200-kernel weight for the F_1 generation of the 30 hybrid test in 1976 and 1977 and the F_2 generation in 1977.

Character	F_1 generation 1976	F_1 generation 1977	F_2 generation 1977
Protein %/yield	-0.11	-0.06	-0.10
Protein %/200 kwt.	0.12	0.18	0.13
Protein %/protein yield	0.46**	0.56**	0.31
Protein yield/yield	0.78**	0.76**	0.88**
Yield/200-kernel weight	0.36*	0.29	0.41*

*,** r_s significant at the 5% and 1% probability levels, respectively.

tein yield were highly correlated, (+0.46** and +0.56** in 1976 and 1977, respectively). Protein yield versus grain yield was even more highly correlated, (+0.78** and +0.76** in 1976 and 1977, respectively).

The yield rank of the top yielding 10 hybrids in 1976 was compared with their rank in 1977 (Table 18). Progenies of five crosses having high protein parents were among the 10 best yielding hybrids in 1976. Four of these progenies remained among the top 10 in 1977.

Table 19 is a summary of parent and F_1 protein levels for hybrids in each of the three groups of crosses. Dominance was in the direction of low protein in almost all cases, the only exception being a low protein cross in which the F_1 progeny showed dominance in the direction of high protein in both years. This hybrid was low yielding, however, most hybrids had lower protein than the low protein parent and all hybrids which fell between the parents in protein level, had a protein percentage near that of the low parent.

Recently, Diehl et al. (1978) reported additive genetic effects in high protein crosses of wheat, although the F_1 means were near those of the low protein parent. Their data did not fit Hayman's 3-parameter model of mean, additive and dominance effects and they suggested that epistasis was involved. They proposed that it would be advantageous to delay selection until the F_3 to increase the frequency of desirable epistatic combinations.

Table 18. Comparison of the yield rank of the top 10 hybrids in 1976 with their rank in 1977 and with parent protein levels.

Yield rank 1976	Parent Protein	Yield rank 1977
1	H x H	3
2	L x L	1
3	H x H	5
4	H x H	9
5	L x L	-
6	H x H	-
7	L x L	-
8	H x L	10
9	H x H	7
10	L x H	2

Table 19. Parental (P_1 and P_2) and F_1 protein percentages in 1976 and 1977.

Group	1976			1977		
	P_1	P_2	F_1	P_1	P_2	F_1
H x H	22.3	23.7	19.9	21.0	20.7	20.0
	22.3	21.4	14.8	21.0	21.4	14.5
	22.5	25.5	17.3	22.2	22.5	19.2
	22.8	24.6	18.7	20.4	23.5	18.9
	22.8	21.4	16.5	20.4	21.4	16.7
	22.0	25.0	17.8	22.9	27.5	18.3
	23.7	22.3	20.5	20.7	21.0	19.8
	25.0	23.0	17.0	27.5	23.8	19.0
	22.0	23.9	18.6	22.9	23.5	18.9
	23.9	23.0	19.4	24.5	19.6	19.3
	24.6	23.2	19.5	23.5	23.8	19.3
	23.9	22.5	18.8	23.5	22.2	19.4
	25.5	23.9	15.3	22.5	23.5	16.8
H x L	15.6	25.7	17.0	16.5	27.5	17.6
	15.7	23.0	15.1	14.4	23.8	14.8
	23.0	18.7	17.9	23.8	19.2	17.5
	23.9	18.7	16.3	23.5	19.2	17.1
	23.2	14.3	15.9	19.6	14.4	15.7
	15.7	23.2	14.5	14.4	19.6	13.5
	24.6	16.9	17.3	24.5	16.1	17.1
L x L	15.6	15.5	16.6*	16.5	14.4	17.0*
	15.6	16.9	13.4	16.5	16.1	14.8
	15.7	16.0	13.3	14.4	14.1	13.5
	13.4	16.0	14.4	12.4	14.1	14.9*
	13.4	17.8	13.1	12.4	17.3	14.5
	16.0	15.7	13.2	14.1	14.4	12.8
	18.7	17.8	14.8	19.2	17.3	15.3
	15.5	15.6	14.7	14.4	16.5	13.1
	16.0	17.8	14.9	14.1	17.3	14.8
	16.9	15.6	14.7	16.1	16.5	14.2

* Dominant in the direction of high protein. All others show dominance in the direction of low protein.

4.3 Six-parent diallel cross

The analyses of variance for the diallel cross data obtained from the Glenlea- and Winnipeg-based experiments of 1977 is shown in Table 20. Location effects were significant only for F_2 protein percentage and F_1 200-kernel weight. It should also be noted that no genotype by location interaction effects were detected for protein percentage, yield in grams per plot, or 200-kernel weight. Therefore, we may assume that the genotypes responded in the same way relative to one another at both locations. Genotypic differences were detected (significant at 0.01 probability level) for percentage protein, yield in grams per plot and 200-kernel weight for both F_1 and F_2 generations.

Further analysis of variance of the data for general (GCA) and specific combining ability (SCA) and for reciprocal effects indicated the presence of highly significant GCA effects for protein percentage, yield in grams per plot and 200-kernel weight in both F_1 and F_2 generations. Specific combining ability effects were significant in the F_1 and F_2 populations for protein percent and in the F_1 for yield in grams per plot. Effects due to SCA were not detected in the F_1 for 200-kernel weight, however, they were present in the F_2. Possibly this is due to differences in inbreeding depression observed in the various hybrids (Table 21). The influence of reciprocal crosses was detected for yield in grams per plot in the F_1 generation and for protein percentage and 200-kernel weight in the F_2

Table 20. Mean squares from the analyses of variance of a six-parent diallel cross grown at Glenlea and Winnipeg in 1977.

Source	df	Character		
		Protein%	Yield	200-kwt.
			F_1 generation	
Location	1	1.66*	5908.0	0.85**
Genotype	29	8.11**	11957.3**	1.23**
GCA†	5	9.79**	6185.9**	1.59**
SCA	9	0.86**	3370.8**	0.04
REC	15	0.14	1479.6**	0.03
Genotype x Location	29	0.30	1632.4	0.07
Error	60	0.48	1948.6	0.14
Total	119			
			F_2 generation	
Location	1	4.21**	21.7	0.01
Genotype	29	12.15**	6176.4**	0.34**
GCA	5	15.54**	7258.3**	0.26**
SCA	9	0.54**	360.9	0.03*
REC	15	0.37**	349.3	0.05**
Genotype x Location	29	0.29	1563.3	0.08
Error	60	0.35	1046.3	0.05
Total	119			

*,** Mean squares significant at 5% and 1% probability levels, respectively.

† GCA, SCA and REC are general combining ability, specific combining ability and reciprocal mean squares, respectively.

Table 21. Percentage inbreeding depression observed from F_1 to F_2 of a six-parent diallel cross of inbred rye lines.

Hybrid	Character		
	Protein (%)	Yield	200-kwt. (g)
A/B	-9.3**	- 1.1	10.5**
A/C	5.9*	41.2**	25.0**
A/D	0.3	18.2*	16.9**
A/E	0.8	3.3	12.4*
A/F	-2.8	35.3**	10.0*
B/C	1.6	16.3*	23.9**
B/D	2.4	30.3**	12.4*
B/E	0.2	14.4*	7.8
B/F	-6.5**	26.8*	3.8
C/D	1.6	51.6**	26.6**
C/E	5.8*	16.3*	20.5**
C/F	4.8	35.7**	27.4**
D/E	0.9	28.9**	6.9
D/F	-7.1**	33.4**	15.0**
E/F	-8.6*	6.9	5.9

*,** Inbreeding depression is significantly greater than 0 at 5% and 1% probability levels, respectively.

generation.

Mean squares for GCA were much higher than mean squares for SCA, suggesting that additive gene effects were predominant for the characters observed.

The GCA effects for the F_1 and F_2 populations were estimated for protein percent, yield in grams per plot and 200-kernel weight (Tables 22 and 23). Two of the parents (B and E) exhibited positive effects for both protein percentage and yield while two parents (C and F) had negative effects on both protein percentage and yield in the F_1 and F_2 generations. Three parents in the F_2 generation (C, D and F) had negative GCA effects on protein percentage, yield and 200-kernel weight. Of the parental lines, C, D and F were the lowest in protein percentage.

Parents A, B and E were the highest in protein percentage. Parent A, although it contributed positively to GCA effects for protein percentage, had a negative effect on yield in both the F_1 and F_2 generations. Table 24 shows parent A to have the highest GCA and SCA variance for yield, however, yields of hybrids with A as a parent are generally low. Further evidence from Table 21 indicates that parent A has an inverse effect on yield and protein percentage as does parent F. Of the five populations exhibiting inbreeding depression, four involve parent F and two involve A (in one population both A and F are involved). In every population, inbreeding depression occurred for kernel weight and in all but one for yield. It would

Table 22. Estimates of general combining ability effects for protein percentage, yield in grams per plot and 200-kernel weight in grams of a six-parent diallel cross of spring rye inbreds (F_1 generation).

Parent	Character		
	Protein	Yield	200-kwt.
A	1.20	-46	0.05
B	0.77	24	-0.45
C	-1.36	- 3	0.84
D	-0.63	32	-0.17
E	0.97	5	-0.04
F	-0.95	-12	-0.23
S.E. g_i	0.11	7	0.06
S.E. $g_i - g_j$	0.17	11	0.09

Table 23. Estimates of general combining ability effects for protein percentage, yield in grams per plot and 200-kernel weight in grams of a six-parent diallel cross of spring rye inbreds (F_2 generation).

Parent	Character		
	Protein	Yield	200-kwt.
A	1.30	-23	0.14
B	1.16	40	-0.10
C	-2.30	-28	-0.05
D	-0.73	- 6	-0.26
E	0.88	35	0.25
F	-0.30	-18	-0.02
S.E. g_i	0.10	5	0.04
S.E. $g_i - g_j$	0.15	8	0.06

Table 24. Estimates of general and specific combining ability variances from F_1 data of a six-parent diallel cross of spring rye inbreds.

Parent	Character	Component GCA	Component SCA
A	Protein %	1.33	0.31
	Yield (g)	1320.10	2018.20
	200-kwt. (g)	-0.01	-
B	Protein %	0.50	0.30
	Yield (g)	985.10	454.50
	200-kwt. (g)	0.19	-
C	Protein %	1.74	1.77
	Yield (g)	1033.60	-93.40
	200-kwt. (g)	0.70	-
D	Protein %	0.30	1.35
	Yield (g)	394.10	918.00
	200-kwt. (g)	0.11	-
E	Protein %	0.84	0.52
	Yield (g)	788.40	-75.70
	200-kwt. (g)	-0.01	-
F	Protein %	0.81	1.84
	Yield (g)	871.00	35.40
	200-kwt. (g)	0.04	-

Table 25. Estimates of general and specific combining ability variances from F_2 data of a six-parent diallel cross of spring rye inbreds.

Parent	Character	Component GCA	Component SCA
A	Protein %	1.66	0.23
	Yield (g)	470.50	-
	200-kwt. (g)	0.02	-
B	Protein %	1.32	0.20
	Yield (g)	1539.40	-
	200-kwt. (g)	0.01	-
C	Protein %	5.28	0.05
	Yield (g)	721.90	-
	200-kwt. (g)	-	-
D	Protein %	0.51	0.12
	Yield (g)	-26.70	-
	200-kwt. (g)	0.06	-
E	Protein %	0.79	0.12
	Yield (g)	1181.40	-
	200-kwt. (g)	0.06	-
F	Protein %	0.07	0.09
	Yield (g)	255.40	-
	200-kwt. (g)	-	-

appear that parents A and F have limited value either in the construction of a synthetic variety or in the production of superior F_1 hybrid combinations.

Parents B and E show somewhat intermediate values for GCA variance for both yield and protein percentage. GCA effects were positive for both parents. Parent B was intermediate for SCA variance and parent E was negative. The relatively higher SCA variance for yield of parent B indicates that certain combinations with B as a parent have higher yield than expected and others, lower yield than expected.

The general combining ability is used to designate the average performance of a line in hybrid combinations. The early literature was reviewed by Sprague and Tatum (1942) and indicated that for yield inbred performance generally was not strongly related to hybrid performance in maize.

On the other hand, specific combining ability is used to describe those cases in which certain combinations do better or worse than expected based on the average performance of the inbred lines involved. Low estimates of GCA variance indicate that the parents are average in their GCA effects, e.g. lines B and D in the F_1 and D and F in the F_2 populations. High estimates of GCA variance indicate that a particular line was much better or poorer than average.

Low estimates of SCA variance indicate that the line performed as expected on the basis of GCA. High estimates of SCA variance appear because some combinations

perform relatively better or poorer than expected on the basis of their average performance. Thus, SCA is attributed to the activity of genes with dominance or epistatic effects.

SCA variances in the F_2 generation were all much smaller than GCA variances; SCA for yield was non-significant. Reduced estimates of SCA compared to GCA should occur if the assumption that SCA is due largely to non-additive gene effects holds. It is also noteworthy that GCA variances for parents B and E are the largest (Table 24). Positive GCA effects for both protein and yield were observed for parents B and E (Tables 22 and 23). The GCA effect for protein percentage was higher in parent B than in parent E, therefore, indicating that parent B contributed more to the average of the hybrids than did parent E. The results of the experiment indicated that the average effects of the inbred lines were much more important than the specific effects of the inbred lines in the determination of hybrid performance.

Sprague and Tatum (1942) compared estimates of the relative importance of GCA and SCA variance in single-crosses of maize, derived from tested and untested inbred lines. They demonstrated that single-crosses of previously untested inbred lines displayed predominantly GCA effects. After testing inbred lines, new material derived (inbred lines), showed an excess of SCA effects over GCA effects. The inbred lines of rye used in this study were previously untested. In light of the results obtained by researchers

in the evaluation of combining ability in maize, it is not surprising that GCA effects were greater than SCA effects in the rye inbreds used.

Morgenstern and Geiger (1975) concluded that most of the genetic variation for yield in rye was due to GCA effects. However, SCA was important in certain crosses. Scoles and Evans (1978) have also suggested that GCA effects may be most important in yield of hybrid rye.

Most of the F_3 lines showed a lack of relationship between protein percentage and 200-kernel weight as was revealed by the non-significant regressions (Table 26). Crosses which show a significant regression tend to involve at least one high protein parent. Regression of seed weight on protein percentage would be expected to indicate a negative relationship. However, of the five significant regressions, three were positive. Without exception, where a significant regression occurred in a given population it did not occur in its reciprocal. Two of the reciprocals tended to be large and of the same sign.

The particular set of inbred lines used in this study transmit their genes for protein content to their progeny in an additive fashion. Due to the predominance of GCA effects, the breeder should first evaluate his material for GCA with the aim of producing an improved synthetic. Because of the additive nature of the genes controlling the protein content it should be possible to select directly for the character. Selection could be practised on open-

Table 26. Regressions of protein percentage on seed weight of F_3 lines derived from single F_2 plants of the six-parent diallel.

Hybrid	b	r	Reciprocal	b	r
A/B	-3.30**	-0.77	B/A	-0.69	-0.39
A/C	-0.23	-0.08	C/A	0.47	0.22
A/D	-0.24	-0.09	D/A	-0.93	-0.24
A/E	-1.38	-0.34	E/A	0.02	0.01
A/F	1.36*	0.50	F/A	-0.32	-0.08
B/C	0.70	0.26	C/B	-0.69	-0.20
B/D	-1.58	-0.27	D/B	-0.53	-0.11
B/E	-0.47	-0.13	E/B	0.74	0.19
B/F	-0.97**	-0.51	F/B	-0.03	-0.01
C/D	-0.11	-0.09	D/C	-0.58	-0.29
C/E	0.88*	0.47	E/C	-0.32	-0.17
C/F	-0.44	-0.19	F/C	0.03	0.02
D/E	0.84	0.37	E/D	0.79*	0.43
D/F	0.53	0.20	F/D	0.14	0.08
E/F	0.78	0.33	F/E	-0.02	-0.01

*,** Significant regression at 5% and 1% probability levels, respectively.

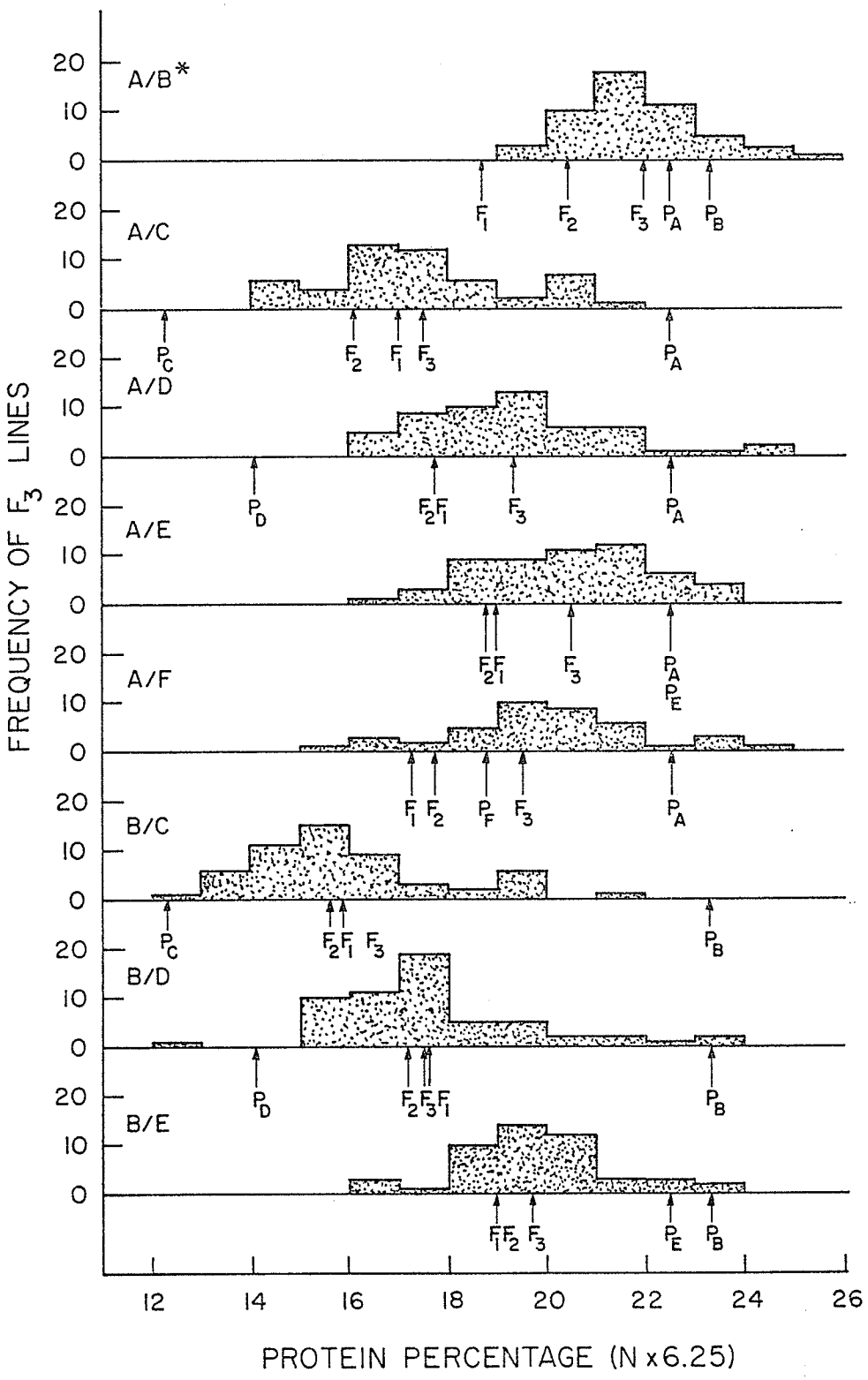
pollinated varieties, population or synthetics.

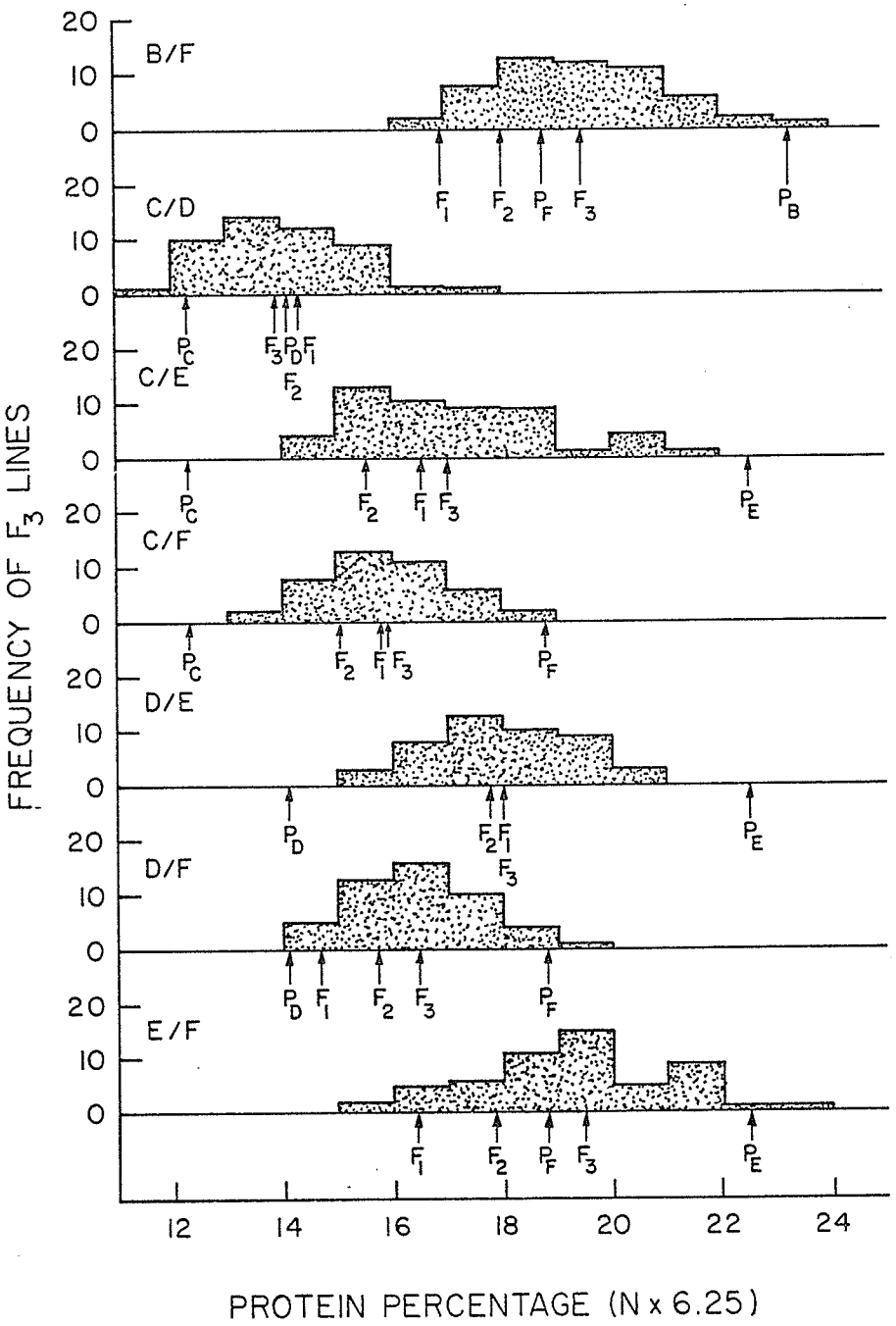
Frequency distributions for protein percentage of bulked reciprocals of the F_3 lines are shown in Figure 3. Means for the F_1 and F_2 generations are indicated also. It can be seen that strong dominance in the direction of low protein was present when both parents had high protein levels, i.e. $P_A \times P_B$, P_E and P_F. Progeny means were near the mid-parent values when high and low protein parents were combined, i.e. $P_A \times P_C$ and P_D.

Figure 2. Frequency distribution in number of F_3 lines derived from F_2 plants (reciprocals bulked), of the various parental combinations of the diallel cross.

* Indicates hybrid combination

\+ Arrows show the mean protein percentage of the F_1, F_2, F_3 and parental generations.


A/B*
A/C
A/D
A/E
A/F
B/C
B/D
B/E
FREQUENCY OF F3 LINES
PROTEIN PERCENTAGE (N x 6.25)
20
10
0
12
14
16
18
20
22
24
26
F1
F2
F3
PA
PB
PC
PD
PE
PF


FREQUENCY OF F3 LINES
B/F
C/D
C/E
C/F
D/E
D/F
E/F
20
10
0
F1 F2 PF F3 PB
PC F3 PD F1 F2
PC F2 F1 F3 PE
PC F2 F1 F3 PF
PD F2 F1 F3 PE
PD F1 F2 F3 PF
F1 F2 PF F3 PE
12 14 16 18 20 22 24
PROTEIN PERCENTAGE (N x 6.25)

GENERAL DISCUSSION

A considerable range of variation has been demonstrated in the present study in the protein levels of spring rye inbred lines (Secale cereale L.). It is surprising that Kotvics (1971) and Focke (1956) proposed interspecific crossing as a means to improve the protein quantity and quality in rye when there seems to be adequate variability within Secale cereale itself as demonstrated in this study and also by Kobyljanskii (1969). When improving protein content, especially in interspecific hybrids, one needs to adjust protein quantity and quality for associated yield effects in order to obtain a true estimate of the relative advantages of the alternate protein sources. The present author's conclusion based on results reported herein is that considerable effort should be made to improve the protein quantity using the inherent variability already within Secale cereale. It is questionable whether the extra effort involved in interspecific hybridization and the subsequent selection for agronomically desirable plant types is justified.

Favret et al. (1969) outlined possible approaches to obtain increased protein quantity and quality. These approaches include, (a) increasing agronomic yield and

percent protein, (b) altering relative proportions of different proteins in the endosperm, and (c) modifying one or more of the proteins toward obtaining a higher frequency of essential amino acids. The results of this study indicate that it may be possible to increase agronomic yield and percentage protein simultaneously in rye. This possibility has been suggested for wheat (Johnson et al., 1973) and oats (Spilde et al., 1974).

Problems occur when selecting inbred lines on the basis of protein content for the synthesis of hybrids in rye. Generally, the protein content of high protein inbreds is greater than that of the hybrids derived from them. When F_1 hybrids are produced the progeny usually exhibit a level of protein which is near that of the low-parent value in rye. This situation would appear at first inspection to be one of partial to complete or overdominance for low protein. This is also documented in the literature for Avena sativa (Ohm and Patterson, 1973; Frey et al., 1954) and Avena sterilis (Sraon et al., 1975). Spilde et al. (1974) observed F_1 progeny to have protein levels intermediate to the parents in interspecific crosses of oats (Avena sativa x A. sterilis). Interspecific crosses showed an additive gene effect whereas intraspecific crosses displayed a dominance relationship.

Dhaliwal (1977) suggested that the partial to complete dominance of low protein over high protein content in wheat and other cereals is the expression of a dominance

relationship between regulatory genes rather than structural genes for seed proteins. Generally, electrophoresis spectra of cultivars and mutants differing in protein content do not differ qualitatively. This suggests that structural genes do not exhibit dominance-recessiveness relationships.

There are many structural genes for storage protein and, thus, the failure of a few of them to express themselves should not produce detectable differences in total protein content. However, regulatory genes tend to be few in number and may exert control over many quantitative structural genes, for example NapHal wheat. Protein content has generally been shown to be under polygenic control of varying complexity (Halloran, 1975). He suggested that a pedigree program would be the most advisable breeding approach for the improvement of wheat protein.

In Section 4.1 of this thesis it was shown that an excess of low protein inbreds existed among the 10 highest yielding lines. However, from two years of testing of F_1 hybrids it was evident that hybrids derived from high protein parents had higher yielding ability as a group of crosses, than did hybrids derived from low protein parents. Therefore, the yield per se of inbred lines would not be useful to predict hybrid yield and some form combining ability test should be used for this character. Similar results were found earlier with maize (Sprague and Tatum, 1942).

Protein yields of the progeny of high protein parents were the highest of the three groups in both years.

This finding is in agreement with the results of Johnson et al. (1973a). Although they were only using two genotypes (high and low protein), the protein yield of the high protein genotype was the highest at all locations and over all levels of nitrogen applied.

One of the major problems encountered with inbreds of rye employed in this study and which may be particular to inbreds developed by self-pollination, was that of inbreeding depression of yield and kernel weight. When F_1 hybrids were self-pollinated, the yield and kernel weight of the F_2 generation was consistently lower than that of the F_1 generation in all populations except one. To reduce the amount of depression that occurs upon inbreeding it may be desirable to make 3-way or double-cross combinations.

Since GCA effects for protein content, yield and 200-kernel weight were greater than those resulting from SCA effects, the production of synthetics may be an alternative to production of hybrids. Inbred lines could be first selected directly for protein level and subsequently progeny-tested for GCA by a topcross test using yield and protein as selection criteria. Superior lines then would be composited to produce a synthetic variety.

During the inbreeding process, lines of rye tend to become more and more self-fertile (data unpublished). This trend could create problems in the development of a synthetic variety as there would be a considerable amount of self-pollinated seed produced. It has been observed

(data unpublished) that when inbred lines of rye developed at the University of Manitoba are polycrossed, up to 50 percent of the polycross population could be identified as the inbred phenotype. For this reason it may be desirable to maintain the self-incompatibility present in most populations of rye. Inbreeding at a slower rate could be achieved by pair sib-matings while concurrently self-pollinating each parental plant of the pair to check for the loss of self-incompatibility. Only those pairs which proved to be self-incompatible would be retained for further selection and testing.

In the present study, there was a lack of significant correlations between 200-kernel weight and protein percentage. The correlation was significant in certain groups of inbred lines in 1975; however, the relationship was non-significant for the inbreds and F_1 hybrids grown in 1976 and 1977, and also for most of the F_3 populations derived from single F_2 plants of the diallel cross. These results are in agreement with those of McNeal et al. (1971). They found non-signficant correlations for 200-kernel weight versus protein percentage in wheat.

The exact basis of increased protein levels in the cereal grains is not known. However, it is well known that increased dosages of nitrogen fertilizer will increase both yield and protein levels. It is also known that certain cultivars inherently accumulate more protein than others. Oudina (1978) reported that differences in seedling

nitrate uptake rate explained 23.8 and 23.6 percent of the variation in grain yield and 22.6 and 19.3 percent of the variation in grain nitrogen for two populations of wheat, respectively. He suggested the possibility of selection for yield based on ion uptake efficiency. However, the potential for selection based on the relationship of nitrate uptake rate to yield and protein has not been fully investigated but would seem to be low, based on the above figures.

A second possible explanation of the variable levels of grain nitrogen (protein percent) is that of variation in the efficiency of nitrogen metabolism and movement of nitrogen to the grain from the plant. This hypothesis has been advanced by McNeal et al. (1971, 1972) for wheat. Both of the above possibilities remain to be examined in rye. Rye has been shown to be different from wheat and barley in the participation of the spike in shoot photosynthesis (Birecka and Zinkiewicz, 1968). The assimilates formed by the spike are only 15 to 29 percent of the final grain yield compared to about 40 percent reported for barley (Thorne, 1963). A proper nitrogen:carbon balance must be maintained at all stages in the development of plants. Eilrich and Hageman (1973) showed that when lodging occurred in wheat, carbon metabolism was reduced and, consequently, less nitrogen was translocated to the grain.

This study has shown that, (a) rye populations can be differentiated into a wide range of protein levels

when they are inbred, (b) F_1 hybrid protein levels can be predicted from parent protein levels, (c) GCA is more important than SCA in predicting the performance of specific rye lines, and (d) the highest protein segregates come from crosses combining high protein parents. These facts indicate that some form of pedigree selection would be effective in increasing protein levels in rye.

Some of the genetic material developed and utilized during the course of this study also may be useful for metabolic studies. High and low protein genotypes may be used to elucidate such aspects of nitrogen metabolism as nitrate uptake, translocation and assimilation of nitrogen into the grain.

CONCLUSIONS

1. Protein content (percent) in rye tends to be negatively correlated with seed weight, although in some cases, the correlation was not significant.
2. Most sources of inbred lines examined could be differentiated into a wide range of protein levels.
3. Inbred sources may be widely differentiated in the S_3 (F_4) or possibly earlier.
4. The S_2 (F_3) lines derived from crosses in the six-parent diallel also showed considerable variation.
5. Selection for protein levels in hybrids can be done on the basis of protein level in the inbred lines.
6. Low protein was partially dominant in hybrids with high protein parents, intermediate in hybrids with high and low protein parents.
7. General combining ability (GCA) effects were greater than specific combining ability (SCA) effects in the small sample of inbreds used in the six-parent diallel.
8. Hybrid combinations were found which yielded greater than or equal to Gazelle, the best commercial spring rye cultivar available in Manitoba. Protein levels were comparatively high in some of the high yielding hybrids and were maintained over two years in tests.

LIST OF REFERENCES

BARLOW, K.K., M.S. BUTTORSE, D.H. SIMMONDS and M. VESK. 1973. The nature of the starch protein interface in wheat endosperm. Cereal Chem. 50: 335-336.

BENNETT, M.D. and J.B. SMITH. 1971. The 4C nuclear DNA content of several Hordeum genotypes. Can. J. Genet. Cytol. 13: 607-611.

BENNETT, M.D. and J.B. SMITH. 1976. Nuclear DNA amounts in angiosperms. Roy. Soc. Lond. B Phil. Trans. 274: 227-274.

BENNETT, M.D., J.P. GUSTAFSON and J.B. SMITH. 1977. Variation in nuclear DNA in the genus Secale. Chromosoma 61: 149-176.

BINGHAM, J. 1961. Some aspects of inheritance of grain quality in wheat. Heredity 16: 237 (Abstr.)

BIRECKA, H. and E. ZINKIEWICZ. 1968. Photosynthesis and ^{14}C-assimilate translocation in winter rye after heading. Bull. Acad. Pol. Sci. Ser. Sci. Biol. 16: 323-329.

BRUNORI, A., P. MANNINO, G. ANCORA and A. BOZZINI. 1977. Protein accumulation, RNA and soluble amino nitrogen content in developing endosperm of two varieties of Triticum aestivum with high and low protein seed. Theor. Appl. Genet. 50: 73-77.

CHEN, C.H. and W. BUSHUK. 1970. Nature of proteins in triticale and its parental species. I. Solubility characteristics and amino acid composition of endosperm proteins. Can. J. Plant Sci. 50: 9-14.

DHALIWAL, H.S. 1977. Genetic control of seed proteins in wheat. Theor. Appl. Genet. 50: 235-239.

DIEHL, A.L., V.A. JOHNSON and P.J. MATTERN. 1978. Inheritance of protein and lysine in three wheat crosses. Crop Sci. 18: 391-395.

EILRICH, G.L. and R.H. HAGEMAN. 1973. Nitrate reductase activity and its relationship to accumulation of vegetative and grain nitrogen in wheat (Triticum aestivum L.). Crop Sci. 13: 59-66.

FAVRET, E.A., R. SOLARI, L. MANGHERS and A. AVILA. 1969. Genetic control of the qualitative and quantitative production of endosperm proteins in wheat and barley. In: New Approaches to Breeding for Improved Plant Protein, IAEA, Vienna, pp. 87-107.

FOCKE, R. 1956. On the crude protein content of S. cereale and S. montanum and the percentage proportion of some amino acids. Züchter 26: 40-41.

FREY, K.J., M.C. SHEKELTON, H.H. HALL and E.J. BENNE. 1954. Inheritance of niacin, riboflavin and protein in two oat crosses. Agron. J. 46: 137-139.

FROST, S. 1963a. Number of accessory chromosomes and protein content in rye seeds. Hereditas 50: 150-160.

FROST, S. 1963b. Pollen fertility, kernel weight and protein content in some inbred lines of rye. Hereditas 50: 335-336.

FROST, S. and S. ELLERSTROM. 1965. Protein content and fertility in tetraploid rye. Hereditas 54: 119-122.

GRIFFING, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. 9: 463-493.

HAGBERG, A.E. and K.-E. KARLSSON. 1969. Breeding for high protein content and quality in barley. In: New Approaches to Breeding for Improved Plant Protein, IAEA/FAO, STI/PUB 212, Vienna, pp. 23-28.

HALLORAN, G.M. 1975. Genetic analysis of grain protein percentage in wheat. Theor. Appl. Genet. 46: 79-86.

JAGANNATH, D.R. and C.R. BHATIA. 1972. Effect of rye chromosome 2 substitution on kernel protein content of wheat. Theor. Appl. Genet. 42: 89-92.

JOHN, P.D.L. and R.N. JONES. 1970. Molecular heterogeneity of soluble proteins and histones in relationship to the presence of B-chromosomes in rye. Experimental Cell Research 63: 271-276.

JOHNSON, V.A., J.W. SCHMIDT and P.J. MATTERN. 1968. Cereal breeding for better protein impact. Econ. Bot. 22: 16-25.

JOHNSON, V.A., A.F. DREIER and P.H. GRABOUSKI. 1973a. Yield and protein responses to nitrogen fertilizer of two winter wheat varieties differing in inherent protein content of their grain. Agron. J. 65: 259-263.

JOHNSON, V.A., P.J. MATTERN, J.W. SCHMIDT and J.E. STROIKE. 1973b. Genetic advances in wheat protein quality and composition. Proc. 4th. Int. Wheat Genet. Symp., Missouri, pp. 547-556.

KHAN, A. HASSAN. 1973. Radiation-induced high-protein mutant strain of wheat. Proc. 4th. Int. Wheat Genet. Symp., Missouri, pp. 273-274.

KOBYLJANSKII, V.D. 1969. Initial material of rye for breeding for grain quality. [Plant Breeding Abstracts 39, #2238]. Selekcija i Semenovodstvo 33(5): 21-23.

KOTVICS, G. 1971. Investigations on increasing the protein content of Secale cereale L. In: Protein Growth by Plant Breeding. Ed. A. Balint. pp. 89-98. [Plant Breeding Abstracts 41, #6920].

KUBICZEK, R. and M. RAKOWSKA. 1974. Characteristics of the nutritive value of the protein from rye caryopsis. I. Amino acid composition of the protein and the nitrogen forms in the caryopsis of ten rye varieties from the breeding collection. Acta Agrobotanica 27: 105-113.

LEE, K.H., R.A. JONES, A. DALBY and C.Y. TSAI. 1976. Genetic regulation of storage protein content in maize. Biochem. Genet. 14: 641-650.

McNEAL, F.H., M.A. BERG, P.L. BROWN and C.F. McQUIRE. 1971. Productivity and quality response of five spring wheat genotypes, Triticum aestivum L., to nitrogen fertilizer. Agronomy J. 63: 908-910.

McNEAL, F.H., M.A. BERG, C.F. McQUIRE, V.R. STEWART and D.E. BALDRIDGE. 1972. Grain and plant nitrogen relationships in eight spring wheat crosses, Triticum aestivum L. Crop Sci. 12: 599-602.

MERTZ, E.T., L.S. BATES and O.E. NELSON. 1964. Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science 145: 279-280.

MIEZAN, K., E.G. HEYNE and K.F. FINNEY. 1977. Genetic and environmental effects on the grain protein content in wheat. Crop Sci. 17: 591-593.

MORGENSTERN, K. and H.H. GEIGER. 1975. General and specific combining ability in testcrosses between inbred lines of rye. EUCARPIA meeting on rye breeding. Poznan, Poland, June 17-21, 1974. Hodowla Roslin Aklimatyzacja i Nasiennictwo 19: 386-390.

MORRIS, R., J.W. SCHMIDT, P.J. MATTERN and V.A. JOHNSON. 1973. Chromosomal locations of genes for high protein in wheat cultivar 'Atlas 66'. Proc. 4th. Int. Wheat Genet. Symp., Missouri, pp. 715-718.

MULLER, F. and G. STERNKOPF. 1976. Uber die Variabilitat de Rohproteinghalts und der Aminosaurengarnitur bei Tetraroggen-Inzuchtlinien. Tag.-Ber. Akad. Landwirtsch. -Wiss. DDR. Berlin 143: 201-210. (English summary).

MUNCK, L. 1964. The variation in nutritive value of barley. I. Variety and nitrogen fertilizer effects on chemical composition and laboratory feeding experiments. Hereditas 52: 1-35.

MÜNTZING, A. 1951. Cytogenetic properties and practical value of tetraploid rye. Hereditas 37: 17-84.

NELSON, O.E., Jr and B. BURR. 1973. Biochemical genetics of higher plants. Ann. Rev. Plant Phys. 24: 493-518.

OHM, H.W. and F.L. PATTERSON. 1973a. A six parent diallel cross analysis for protein in Avena sterilis L. Crop Sci. 13: 27-30.

OHM, H.W. and F.L. PATTERSON. 1973b. Estimation of combining ability, hybrid vigour and gene action for protein in Avena spp. L. Crop Sci. 13: 55-58.

OSBORNE, T.B. 1907. The proteins of the wheat kernel. Carnegie Inst. Wash., Publ. No. 84.

OUDINA, M. 1978. Variability in nitrate and phosphate uptake rates in wheat cultivars. M.Sc. Thesis, University of Manitoba.

PAEZ, A.V. 1973. Protein quality and kernel properties of modified opaque-2 endosperm corn involving a recessive allele at the sugary-2 locus. Crop Sci. 13: 633-636.

PANDEY, S. and E.T. GRITTON. 1976. Observed and predicted response to selection for protein and yield in peas. Crop Sci. 16: 289-292.

PLARRE, W. and V. FISCHER. 1975. Analysis of a diallel cross in inbred lines of rye. EUCARPIA meeting on rye breeding. Poznan, Poland, June 17-21, 1974. Hodowla Roslin Aklimatyzacja i Nasiennictwo 19: 391-401.

RILEY, R. and J.A.D. EWART. 1970. The effect of individual rye chromosomes on the amino acid content of wheat grains. Genet. Res., Camb. 15: 209-219.

SCOLES, J.G. and L.E. EVANS. 1978. Hybrid vigour obtained by crossing inbred lines of spring rye. Can. J. Plant Sci. 58: 745-751.

SHEPHERD, K.W. 1968. Chromosomal control of endosperm proteins in wheat and rye. Proc. 3rd. Int. Wheat Genet. Symp., Australian Academy of Science, Canberra, pp. 86-96.

SIGURBJORNSSON, B. 1970. Role of plant breeding in bridging the protein gap. In: Improving Plant Protein by Nuclear Techniques. IAEA, Vienna, pp. 13-17.

SIMMONDS, D.H., K.K. BARLOW and C.W. WRIGLEY. 1973. The biochemical basis of grain hardness in wheat. Cereal Chem. 50: 553-562.

SIMMONDS, D.H. and W.P. CAMPBELL. 1976. In: Rye: Production, Chemistry and Technology. W. Bushuk (ed.). American Association of Cereal Chemists, Inc., St. Paul, Minnesota, p. 88.

SINGH, L. and H.H. HADLEY. 1972. Maternal and cytoplasmic effects on seed protein content in Glycine max L. Merrill. Crop Sci. 12: 583-585.

SINGH, R. and J.D. AXTELL. 1973. High lysine mutant gene (hl) that improves protein quality and biological value of grain sorghum. Crop Sci. 13: 535-539.

SINGH, A.N. and J.S. NANDA. 1976. Effect of the genotype of seed and sporophyte on the protein content of seed in bread wheat (Triticum aestivum L.). Cereal Res. Comm. 4: 51-54.

SPILDE, L.A., R.S. ALBRECHTSEN and M.D. RUMBAUGH. 1974. Relationship of protein percent with other phenotypic characters in interspecific oat crosses. Crop Sci. 14: 767-769.

SPRAGUE, G.F. and L.A. TATUM. 1942. General versus specific combining ability in single crosses of corn. Am. Soc. Agron. J. 34: 923-932.

SRAON, H.S., D.L. REEVES and M.D. RUMBAUGH. 1975. Quantitative gene action for protein content in oats. Crop Sci. 15: 668-670.

STUBER, C.W., V.A. JOHNSON and J.W. SCHMIDT. 1962. Grain protein content and its relationship to other plant and seed characters in parents and progeny of a cross of Triticum aestivum L. Crop Sci. 2: 506-508.

THORNE, G.N. 1963. Varietal differences in photosynthesis of ears and leaves of barley. Ann. Bot. N.S. 27: 155-175.

WAINES, J.G. 1973. Chromosomal location of genes controlling endosperm protein production in Triticum aestivum cv. Chinese Spring. Proc. 4th. Int. Wheat Genet. Symp., Missouri, pp. 873-877.

WALBOT, V. 1973. RNA metabolism in developing cotyledons of Phaseolus vulgaris. New Phytol. 72: 479-483.

WHITEHOUSE, R.N.H. 1970. The prospects of breeding barley, wheat and oats to meet special requirements in human and animal nutrition. Proc. Nutr. Soc. 29: 31-39.

WHITEHOUSE, R.N.H. 1973. The potential of cereal grain crops for protein production. In: The Biological Efficiency of Protein Production. Ed. J.G.W. Jones, Cambridge University Press, pp. 83-99.

WILLIAMS, P.C. 1966. Reasons underlying the variations in the protein content of Australian wheats. Cereal Sci. Today 11: 332-335.

WOLSKI, T., J. BRYKCZYNSKI and E. TYMIENIECKA. 1972. Heritability of some characteristics of rye under open pollination. Theor. Appl. Genet. 42: 168-173.

WORZELLA, W.W. 1934. The inheritance of quality in Trumbull and Michikof varieties of winter wheat. J. Agr. Res. 49: 705-714.

WRICKE, G. 1973. Inzuchtdepression und Genwirkung beim Roggen (Secale cereale). Theor. Appl. Genet. 43: 83-87. (English summary).

YCAS, M., M. SUGITA and A. BENSAM. 1965. A model of cell size regulation. J. Theor. Biol. 9: 444-470.

YOUNGS, V.L. and D.M. PETERSON. 1973. Protein distribution in the oat (Avena sterilis L.) kernel. Crop Sci. 13: 365-367.

YONG, F.C. and A.M. UNRAU. 1966. Alien genome combinations and influence on amino acid composition of cereal protein fractions. J. Agr. Food Chem. 14: 8-12.

APPENDIX

Appendix Table 1. Means of F_1 hybrids from the six-parent diallel cross for protein yield and kernel weight.

Hybrid	Character		
	Protein %	Yield (g)	200-kwt. (g)
A/B	18.71	208.0	6.40
A/C	17.01	250.0	7.53
A/D	17.84	199.8	6.39
A/E	18.95	191.8	6.75
A/F	17.26	242.3	6.49
B/C	15.85	233.0	7.11
B/D	17.60	305.5	6.10
B/E	18.96	331.9	6.14
B/F	16.95	291.9	5.81
C/D	14.34	316.0	7.19
C/E	16.53	253.5	7.41
C/F	15.84	212.0	7.49
D/E	18.00	309.3	6.61
D/F	14.69	273.1	6.40
E/F	16.44	209.9	6.28
S.E.*	.35	22.1	.19
S.E.**	.49	31.2	.27

* Standard error of any F_1 mean value.

** Standard error of the difference between any two F_1 means.

Appendix Table 2. Means of F_2 hybrids from the six-parent diallel cross for protein yield and kernel weight.

Hybrid	Character		
	Protein %	Yield (g)	200-kwt. (g)
A/B	20.45	210.2	5.73
A/C	16.01	147.1	5.65
A/D	17.78	163.4	5.31
A/E	18.80	185.4	5.91
A/F	17.74	156.9	5.84
B/C	15.60	195.0	5.41
B/D	17.18	213.0	5.34
B/E	18.99	284.1	5.66
B/F	18.05	213.6	5.59
C/D	14.11	152.9	5.28
C/E	15.57	212.2	5.89
C/F	15.08	136.4	5.44
D/E	17.84	219.9	5.49
D/F	15.73	181.9	5.44
E/F	17.85	195.5	5.91
S.E.*	.30	16.2	.12
S.E.**	.42	22.9	.17

* Standard error of any F_2 mean value.

** Standard error of the difference between any two F_2 means.

Appendix Table 3. Estimates of Test means for protein percentage, yield in grams per plot and 200-kernel weight for the F_1 and F_2 generations of a six-parent diallel cross.

Generation	Characters		
	Protein %	Yield g/plot	200-kwt. (g)
F_1	17.00	255.2	6.67
F_2	17.13	191.2	5.57

Appendix Table 4. Estimates of CV's for protein percentage, yield in grams per plot and 200 kernel weight for the F_1 and F_2 generations of a six-parent diallel cross.

Generation	Characters		
	Protein %	Yield g/plot	200-kwt. (g)
F_1	4.08	17.3	5.74
F_2	3.45	16.9	4.33

Appendix Table 5. Estimates of specific combining ability for protein and yield in the F_1 generation of a six-parent diallel cross of spring rye inbreds.

Hybrid	Characters	
	Protein %	Yield (g)
A/B	-0.25	-25
A/C	0.17	44
A/D	0.27	-41
A/E	-0.22	-22
A/F	0.02	45
B/C	-0.56	-43
B/D	0.46	-5
B/E	0.22	48
B/F	0.13	25
C/D	-0.67	32
C/E	-0.09	-4
C/F	1.15	-29
D/E	0.66	17
D/F	-0.73	-2
E/F	-0.58	-39
S.E. $s_{ij}-s_{ik}$	0.30	19
S.E. $s_{ij}-s_{kl}$	0.24	16

Appendix Table 6. Estimates of specific combining ability for protein percentage and 200-kernel weight in the F_2 generation of a six-parent diallel cross of spring rye inbreds.

Hybrid	Characters	
	Protein %	200-kwt. (g)
A/B	0.87	0.11
A/C	-0.06	-0.02
A/D	0.08	-0.15
A/E	-0.50	-0.05
A/F	-0.39	0.12
B/C	-0.38	-0.01
B/D	-0.38	0.12
B/E	-0.17	-0.06
B/F	0.07	-0.17
C/D	0.02	0.01
C/E	-0.13	0.11
C/F	0.55	0.10
D/E	0.66	-0.08
D/F	-0.37	0.10
E/F	0.14	0.07
S.E. $s_{ij}-s_{ik}$	0.26	0.10
S.E. $s_{ij}-s_{kl}$	0.21	0.09

Appendix Table 7. Estimates of reciprocal differences in F_1 yield, F_2 protein percentage and F_2 200-kernel weight for a six-parent diallel cross.

Hybrid	Characters		
	F_1 yield (g)	F_2 protein %	F_2 Kwt. (g)
A/B	37.00	.225	.175
A/C	32.00	.088	.200
A/D	14.25	.125	.113
A/E	1.75	.825	.425
A/F	9.25	.038	.113
B/C	10.00	.250	.063
B/D	16.50	.150	.063
B/E	12.38	.313	.038
B/F	1.68	1.150	.213
C/D	1.50	.213	.050
C/E	44.50	.075	.188
C/F	25.25	.100	.088
D/E	52.50	.288	.088
D/F	49.38	.100	.263
E/F	8.38	.275	.013
S.E. r_{ij}	15.61	.209	.085