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The nature of the genetic variation associated with a cross involving winter and spring wheat parents for four quality factors was evaluated. Yamhill, a soft white winter wheat, and Inia 66, a hard red spring wheat, were selected as parents for this study. They represented distinctly different phenotypes for the attributes measured. Parents, F_1, F_2 and reciprocal backcrosses were examined for protein and lysine content, kernel hardness, and sedimentation value.

Significant differences were observed between the two parents for protein content. The F_1 mean was lower than the low protein parent with the F_2 mean intermediate between the two parents. This latter factor plus a high narrow sense heritability estimate suggest that the genetic variation associated with protein content was largely due to genes which act in an additive manner. Backcrosses to either parent shifted the population toward the mean of the recurrent parent. Transgressive segregation was observed in the F_2 for both low and high protein content suggesting that selection for this trait should be effective in early generations.

Parents differed significantly for lysine content with the winter parent, Yamhill displaying lysine values approaching the highest previously reported for wheat. Intermediate F_1 and F_2 population means and a high narrow sense heritability estimate suggest the genes involved function in an additive manner. The backcross progeny to Yamhill had a mean value approaching that of the recurrent parent. No transgressive segregation was observed in the F_2 for lysine content higher than Yamhill. It appears that lysine content in this cross is qualitatively inherited and that selection for improved lysine content above Yamhill appears limited.

The genetic variation associated with kernel hardness appears to be largely additive with F_1 and F_2 means intermediate between the two parents and a high narrow sense heritability estimate. Transgressive segregation was observed in the F_2 generation for both soft and hard kernel types. Selection for this trait should be effective in early generations.

Significant differences were observed for the parental types for sedimentation value. The F_1 and F_2 means were below the midparent value. No transgressive segregation was observed in the F_2 for either low or high sedimentation value. One backcross to the low sedimentation parent brought the population back to the low parent mean. One backcross to the high sedimentation parent shifted the population toward the recurrent parent but no individuals were recovered that approached the high parent. From this study it appears that selection for high sedimentation value types would be very difficult.

Evidence provided in this study supports the concept that winter x spring crosses can provide useable genetic variation for desirable quality factors.

Evaluation of Four Quality Factors in a Selected Winter X Spring Wheat Cross (Triticum aestivum Vill., Host)

by

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Typed by Kathie Klahn for Karen Sue Schumaker

IN DEDICATION TO

My Husband, Mother and Father

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EVALUATION OF FOUR QUALITY FACTORS IN A SELECTED WINTER X SPRING WHEAT CROSS (*Triticum aestivum* Vill., Host)

I. INTRODUCTION

The concept of hybridizing winter x spring wheats is not a new one. Classical varieties such as Thatcher, Federation and many of the cultivars which gave rise to the so-called "Green Revolution" have resulted from winter x spring crosses. What is new, however, is the concept of systematically sharing desired attributes found in the winter and spring type cultivars. This approach has been prompted by concerns that future yield improvement and broadening of cultivar adaptation would be minor if scientists were only to utilize intra winter or spring germ plasm. By crossing winter and spring type cultivars which have been developed under different environments, the breeder is combining a diverse array of germ plasm and thus potentially broadening the base of genetic variability. Many of the lines which have resulted from winter x spring hybridizations show improved levels of disease resistance, wider adaptation and drought tolerance which exceed either parent.

Once the breeder began to extensively use these divergent crosses, concerns were expressed as to what impact such genetic diversity might have on the nutritional quality of the end product. With the projected increase in the role wheat will have to play as the world's population continues to expand, it is essential to improve the nutritional quality of wheat protein. The need is especially critical for those people who have limited access to animal sources of protein. Therefore,

the breeder using winter x spring crosses must be able to maintain wheat's nutritional quality if not improve it.

It was the objective of this study to evaluate the amount of genetic variation present for protein content, lysine content, kernel hardness and sedimentation (gluten strength) resulting from a winter x spring cross. Parents selected included Yamhill, a low protein, soft white winter wheat and Inia 66, a high protein, hard red spring wheat. Progeny analyzed included the F_1, F_2 and the backcrosses to the respective parents. Information was obtained for the following factors: percent protein, lysine, kernel hardness and sedimentation. From these data the nature of the inheritance of factors which control these quality characters was determined.

II. LITERATURE REVIEW

Protein

Wheat provides nourishment for more people than does any other food source (Inglett and Anderson, 1974), and it is expected that in the future it will assume an even greater importance as a source of both carbohydrates and protein for much of the world (Konzak, 1977). Genetic improvement of wheat to better meet human needs would have important and widespread consequences. Enhanced value of wheat as a food encompasses three factors, yield of grain, protein content and the nutritional value of the protein (Johnson et al, 1972). Nutritional value of the wheat protein involves the balance of essential amino acids (Nelson, 1970).

The average protein content of hard wheat usually exceeds 12% and is higher than the protein content of rice, maize or sorghum. In the United States wheat protein values range from 7 to 18% (Johnson et al, 1972). Results from the University of Nebraska research indicate that substantial genetic variation for grain protein content exists in wheat as lines selected from crosses between high protein cultivars were found which had 5% higher protein in their grain than commercially grown varieties (Johnson et al, 1970).

Crop growth environment is thought to modify genetically controlled protein characteristics. Some of the factors thought to influence protein content include nitrogen availability, water supply, temperature, light intensity and photoperiod. The work of Finney and Fryer (1958), Hehn and Barmore (1965) and Kolderup (1975) illustrate the importance

of these factors to protein content. Vogel et al (1976) found that differing maturities and degree of adaptation to the production environment resulted in variability in seed size and plumpness, thereby accounting for much of the variability in protein encountered. Johnson et al (1968) found that a single wheat genotype could produce grain protein varying from 8 to 18% depending on the environment in which it was grown. Terman et al (1969) studied hard red winter wheat cultivars for yield and protein as affected by nitrogen and moisture at several locations. Protein content was found to vary more between locations than among varieties at each location.

There has been a question as to whether protein content is affected by grain yield. The majority of the studies report a negative association between yield and protein (Clark, 1926; Stuber et al, 1962). Terman et al (1969) found a highly negative relationship between yield and protein for several varieties grown under different nitrogen and moisture regimes. Davis et al (1961) evaluated four crosses and found the negative relationship in three out of four populations. Similar results were reported by McNeal et al (1972) when studying crosses of eight spring wheat cultivars. Haunold et al (1962) studied the association of four cultivars over two years and reported inconsistent findings which were influenced by year and variety. No negative correlation between grain protein and yield was reported by Schmidt (1958) and he hypothesized that the two factors are negatively correlated when soil nitrogen is the limiting factor for growth.

The nutritional value of wheat, like the other cereals, is primarily limited by the amount and balance of four essential amino acids.

These four amino acids include lysine, threonine, isoleucine and methionine (Kasarda, 1971). Lysine is in shortest supply in cereal proteins with no known wheat cultivar having a protein composition approaching the desired levels (Schmidt et al, 1974).

Practical Measurement of Protein Content

Quantitative expression of total protein is related to total organic nitrogen in the flour. Protein quantity is measured most often by the Kjeldahl nitrogen analysis which assumes a constant relationship between total nitrogen and the polymers of amino acids which link to form proteins. In wheat flour this relation is expressed by multiplying nitrogen content by 5.7 (AACC, 1962; Crooke and Simpson, 1971). A second method is known as dye binding capacity (DBC). This method uses a solution of acid diazo dye which is mixed with the wheat flour. The dye is bound quantitatively by the basic imidazole, guanido and amino groups of protein which occur in the polypeptide chain on histidine, arginine and lysine. The unbound dye remaining in the solution is measured colorimetrically after filtration or centrifugation. Since the proportions of basic amino acids and terminal groups are reasonably constant in cereal proteins, the correlation between DBC and total protein content is high (Mossberg, 1969). Munck (1970) developed a method to study the adhesion of starch to protein. A sample is taken from the endosperm, placed on a slide and stained with acrilane orange solution. Protein can also be analyzed with the use of a near-infrared reflectance analyzer (NIR) (Williams, 1975). This method relates the intensity of the diffuse surface reflectance, at specific wave lengths, to the composition of the sample. The various

components of a given product have specific near-infrared absorption bands which will affect the reflectance of the sample as the concentration of each constituent changes. To calibrate the machine, readings at multiple wavelengths are taken on a set of Kjeldahl based calibration samples and then combined with the laboratory results using multiple linear least squares regression. The calibration constants obtained from the regression are stored in the instrument and used to determine the composition of unknown samples by solving the equation:

$$F_1 \log R_1 + F_2 \log R_2 + F_3 \log R_3 + F_0 = \% \text{ constituent}$$

where F equals the constant from regression (F value) and log equals the logarithm of reflectance (log values).

Inheritance of Protein Content

Varietal differences in grain protein content have long been known to exist. Johnson et al (1973) screened the United States Department of Agriculture (U.S.D.A.) World Wheat collection and found varieties with protein content ranging from 6 to 22%.

Early investigation into the inheritance of protein (Clark, 1926) suggested that it was inherited as a complex trait. Brock and Langdridge (1973) reported that in the legume and cereal species they examined (spring and winter types) the amount of seed protein was under multigenic control. Munck et al (1970) and Mixra et al (1972) reported that mutants which have altered amino acid composition and thus nutritional quality of protein are under the control of major genes. In their work, Brock and Langdridge (1973) analyzed F_2 families and heritability of protein content was calculated in a number of intervarietal crosses. Their analysis showed that based on protein estimated as the

absolute amount of protein in 100 seeds heritability of protein content was high (70%). They concluded that one or a few major genes were involved in determining a large part of the protein content in wheat kernels. Lebsock et al (1964) examined variation in protein values for parents, F_1's, F_3, F_5 and F_6 generations of the spring wheat cross, PI 56219 x Conley. They concluded that low protein was partially dominant to high protein. Their F_3 lines were normally distributed over the range represented by the parents and the heritability estimates for protein ranged from 37 to 70%. Kaul and Sosulski (1965) studied the inheritance of flour protein in a cross between two hard red spring wheats, Selkirk and Gabo. They studied parents, F_1, F_2, BC_1 and BC_2 generations, and they concluded that there was no net dominance for low or high protein content. Their heritability estimates ranged from 66 to 82%. Chapman and McNeal (1970) crossed two spring wheat high protein parents and found F_1 and F_2 means below the lower protein parent indicating dominance for low protein. Pepe et al (1976) hybridized two hard red semidwarf wheats and 126 F_5 progeny lines were studied to determine the relationship among the variables of plant height, yield and percent protein. They concluded that plant height had no affect on grain yield but found an inverse relationship between yield and percent protein. Since the first report of its high protein level, Atlas 66 (a soft red winter wheat) has been used to investigate the inheritance of protein. Sunderman et al (1965) examined 66 F_2 and F_3 progeny from the cross Itana x Atlas 66 for protein content. Heritability estimates were reported to range from 15 to 26%. Johnson et al (1969) found protein increases from 15 to 25% without reduction in productivity using

Atlas 66 as a parent. High protein from Atlas 66 is reported to be inherited as an incompletely dominant trait with more than a single gene involved. The ease with which high protein lines were recovered from crosses of Atlas 66 with Comanche and Wichita (hard winter wheats) suggested operation of a relatively small number of genes. In 1972 Johnson et al looked at F_2 bulks resulting from a cross between two high protein parents. The F_2 bulks were grown in the F_3 and F_4 generations successively. Protein content of the parent varieties exceeded 20% both years. Mean protein of the F_3 and F_4 populations approximated the midparent value. Transgressive segregation was seen in both the F_3 and F_4 populations. F_2 bulks were analyzed from crosses between high and low protein parents with results suggesting partial dominance of low protein. Solen (1973) looked at parents, F_1's and F_2's in a series of winter x winter crosses. Protein values were intermediate for F_1's and F_2's. For crosses involving Hyslop (a low protein, soft white winter wheat) the mean values were near the high parent and little or no transgressive segregation was seen in the F_2 populations. Diehl et al (1978) studied protein in a cross between two high protein parents. Dominance effects for low protein were detected in all crosses. F_1 means were near or below the low protein parent. F_2 mean values for protein approximated the midparent values suggesting additive gene action. They concluded several genes were involved, with high protein inherited by accumulation of favorable recessive genes with some epistatic gene action.

Lysine

In many countries of the world animal based proteins constitute only a negligible portion of total protein intake either due to lack of availability or cultural (religious) reasons. In these areas, alternative sources of protein become increasingly important with special pressure on cereals. The utilization of cereals as a protein source often leads to malnutrition due to the imbalance in the availability of amino acids (Thielebein, 1969). Protein utilization is limited by the amino acid in shortest supply and lysine is the most limiting essential amino acid in the majority of the cereals. In countries such as India cereals constitute 60% of the protein supply with pulses and nuts 26% (Swaminathan, 1969). Therefore, increased protein content, especially improving the amino acid balance of cereals would make a substantial contribution to alleviating the world protein problem.

Genetic variability for lysine in wheat grain has been found to be limited. Johnson (1977) reported that the genetic component of total lysine variability among 12,600 wheats in the World Wheat Collection was only 0.5%. It has been estimated that increases of 1.5% would be required to bring lysine into reasonable balance with other essential amino acids.

Lysine per unit protein has been shown to be negatively correlated, however, lysine per unit weight of grain has shown a positive correlation (Johnson et al, 1962). This suggests that increasing the protein content of wheat can effectively increase the amount of lysine in the grain on a per weight of grain basis.

Lysine values for the United States Department of Agriculture

(U.S.D.A.) World Wheat Collection samples ranged from 2.2 to 4.2% with a mean of 3.2% for lysine expressed as percent of protein. The majority of the values were in the 2.8 to 3.6% range. Genetic variability for lysine in wheat is thought to be limited but of sufficient magnitude to be useful in breeding. It is thought that lysine may be increased by 0.5 to 0.7% (Johnson et al, 1977). Johnson et al (1972) hypothesize that their inability to measure differences in lysine content may be due to the presence of more than one genome. A gene in one genome with a large effect on lysine could be masked by genes in other genomes. They point out that those species in which genes with large effects on lysine have been identified (corn and barley) are diploid species.

Mutations opaque-2 and floury-2 in maize alter the amino acid composition of seeds. The content of lysine is higher in the mutant than the normal maize lines and this results in higher nutritive value of the mutants. In 1968 Munck et al discovered a high protein, high lysine barley, Hiproly. This discovery makes barley a potentially valuable protein source for the human diet and a potentially low cost livestock feed. So far no high lysine mutants have been discovered in wheat.

Swaminathan et al (1969) report that lysine content responds much the same way as protein to the environment. In their studies with nitrogenous and phosphatic fertilizers they found that lysine content was not influenced by nitrogen when calculated on a flour basis although it showed a declining trend when calculated on a protein basis. Johnson et al (1970) tested the stability of high lysine valued lines by growing the wheats in different environments. Most high lysine wheats failed

to maintain high values originally assigned from laboratory analysis of the World Collection. They concluded, therefore, that the lysine values are largely non-genetic.

Perhaps the most important factor influencing lysine is protein content. The relationship between lysine per unit protein and protein level is negative and nonlinear (Vogel et al, 1973). Increases in grain protein to 15% are associated with severe depression of lysine per unit of protein. Above 15% protein levels there is little depression of lysine. It is hypothesized that the reason for this depression is the fact that high protein wheats contain lower proportions of the lysine rich components of protein than do low protein wheat grains. The depression of lysine per unit increase in protein is not directly proportional. Lysine expressed as percent dry grain weight is positively correlated with protein content. The relationship is mildly curvilinear and is pronounced at the high protein levels. The contribution of protein to lysine per unit weight of grain compensates for the tendency for high protein to be associated with depressed lysine content of the protein (Johnson, 1962).

Practical Measurement of Lysine

Interest in the development of high lysine wheats has led to specialized methods which measure lysine, argenine, histidine or lysine alone (Mattern et al, 1968). Some of the commonly used methods include the following.

The presence of normally high levels of free amino acids may be regarded as indicative of above average levels of lysine. This conclusion is based on the reaction of high lysine mutants (opaque-2 maize). In this method ninhydrin reacts with the alpha amino groups of free

amino acids to form a colored complex whose intensity is proportional to the concentration of free amino acid (Mertz et al, 1974; Mattern et al, 1970).

Lysine decarboxylase is used to measure the amount of lysine and is based on the colorimetric measurement of CO_2 which is specifically released from lysine by the enzyme lysine decarboxylase (Wall and Gehrke, 1974).

Villegas and Mertz (1971) proposed a method for measuring lysine in maize known as colorimetric determination of lysine by 2-chloro-3, 5-dinitro pyridine in papain hydrolyzed maize protein. Lysine and tryptophan are present in a roughly 4:1 ratio. When tryptophan is determined an estimate of lysine is furnished. Papain releases the amino acids and tryptophan plus a combination of reagents (DNP) combine to form glyoxylic acid. Helm (1972) used the same method on soluble protein portions for determining the amount of lysine in barley.

Lysine can also be determined by UDY Orange G dye (disulfonic acid dye). Since the proportions of basic amino acids and terminal groups in the polypeptide chain are reasonably consistent in cereal proteins, correlation between DBC and total protein is high. The dye is bound quantitatively by the imidazole, guanido and amino groups of the protein and the unbound dye remaining is measured colorimetrically. From the results the amount of lysine can be calculated (Mossberg, 1969).

Lysine in wheat grain can also be determined by the use of near-infrared reflectance (NIR). The method is based on lysine values obtained by standard amino acid analysis with automated ion-exchange chromatography (AAA). The near-infrared reflectance analyzer is

calibrated with samples representing a wide range of lysine values. In studies with hard red spring and winter wheats, Rubenthaler and Bruinsma (1978) found lysine values were predicted independently of protein and that correlations for NIR lysine values and AAA lysine values were significant ($r = 0.975$). NIR has been shown to be an economical, simple, rapid and non-destructive method for determining lysine values in wheat grain.

Inheritance of Lysine

Transgressive segregation for lysine in the F_2 generation from a cross involving two high lysine parents (Nap Hal x CI 13449) was reported by Johnson et al (1970). Lysine analysis of F_4 and F_5 selections substantiated previous evidence for transgressive segregation. Studies from a cross involving Hiproly showed that the F_1 progeny were lower in lysine than the Hiproly parent. The F_2 segregated in a 1:3.2 ratio. Munck (1970) hypothesized that there is a recessive major gene influencing lysine synthesis in the seed. In 1971 Munck reported that additional genetic factors could interact with the recessive major gene influencing lysine synthesis. The inheritance of lysine in three wheat crosses (Nap Hal, Atlas 66 and April Bearded) was examined by Diehl et al (1978). They found lysine as percent of protein is inherited primarily by additive gene effects and suggested that lysine is inherited by an accumulation of favorable recessive genes.

Hardness

Hardness and softness are milling characteristics relating to the way the endosperm breaks down. Fragmentation of endosperm in hard wheats is along the lines of cell boundaries while in soft wheats the

endosperm fractures randomly. The hard wheats have areas of mechanical strength and weakness whereas soft wheats are found to be fairly uniform in weakness. Hardness is believed to be related to degree of adhesion between protein and starch (Kent, 1975). Hard wheats yield coarse, gritty flour which is easily sifted whereas soft wheats give very fine flour which adheres together, sifts with difficulty and often clogs sieves. Mechanical damage to starch granules produced during milling is greater for hard wheats than for soft wheats. In hard wheats the endosperm cells come away more cleanly and remain more intact whereas in soft wheats part of the endosperm is left attached to the bran (Kent, 1975).

Unusually hard or soft milling characteristics will often be reflected in lower flour yields. A wheat which is too hard will require more power and more than the normal number of break and reduction operations. If the wheat is too soft it will tend to ball up and not pass through the sieves or will require more sifting time (Davis et al, 1961).

High protein flours are preferable for bread whereas low protein flours are suitable for pasteries. The soft wheats generally have weak gluten traits and short-time mixing properties quite unlike the hard endosperm, strong gluten bread wheats (Reitz, 1964).

Practical Measurement of Kernel Hardness

Anderson et al (1966) have reviewed various methods for determining kernel hardness. For grading purposes kernel hardness is often measured by determination of pearling index. Pearling index equals the percentage of material pearled off from a sample of wheat of prescribed weight in a

laboratory barley pearler operated for a prescribed period of time (Taylor et al, 1939). Beard and Poehlman (1954) studied segregates from hard x soft wheat crosses and found wide differences in pearling indices. The particle size index test is also used to determine kernel texture. In this procedure the sample is ground and a subsample is placed on a nest of sieves of various mesh sizes. The sieves are shaken for five minutes and the flour in the pan is weighed. The index is expressed as the percent yield of flour (Yamasaki, 1972).

Hardness values for wheat kernels may be determined with the use of a near-infrared reflectance analyzer as outlined by Bruinsma and Rubenthaler (1979). They devised a hardness index based on the fact that as hardness index increases the percentage of large particles in the sample will also increase. Correlations for this method and particle size index values were very high ($r = 0.952$).

Inheritance of Kernel Hardness

Symes (1965) ran baking tests on a number of near isogenic wheat lines and found no indication of association of kernel hardness with protein content of grain. He did find that milling extraction, loaf texture and dough handling properties were associated with grain hardness. Similar results were reported by Baker and Dyck (1975) in two spring wheats. A study of the particle size index differences between Falcon (a hard wheat) and Heron (a soft wheat) showed that the difference was due to a single major gene (Symes, 1969). He reported the existence of minor genes which modify the action of major genes in determining kernel hardness or softness. Beard and Poehlman (1969) crossed Kawvale (a semihard wheat) and Pawnee (a hard wheat) with several soft parents.

They found that segregates varied widely in pearling index with most found in between the soft and hard parent. They concluded that kernel hardness is inherited multigenically and is dominant. Baker (1977) used an inbred backcross technique to study the inheritance of kernel hardness in two spring wheat crosses. The crosses involved Pitic 62 (a soft wheat) and Glenlea (a very hard wheat) each crossed to Neepawa (a hard wheat). Second backcrosses were inbred for four generations and grinding time was used as a measure of kernel hardness. He concluded that the difference in hardness of Pitic 62 and Neepawa was governed by two major genes and one or more minor genes and that a single major gene and one or more minor genes accounted for the difference in kernel hardness between Glenlea and Neepawa.

Sedimentation

When wheat flour is mixed with water it forms an elastic substance called gluten. Gluten characteristics of wheat flour account for its ability to make aerated bread.

Practical Measurement of Sedimentation

The quality of wheat protein may be investigated by washing out the gluten and examining its quantity, physical characteristics and color. Often the gluten is examined by swelling of the flour particles in a dilute lactic acid solution (Sedimentation Test, Zeleny, 1947).

The sedimentation test is a simplified water retention capacity test in the presence of acid. Glutens are hydrated and swell upon contact with the lactic acid. It was originally suggested that sedimentation measured both protein content and protein quality. Muller (1970,1973) reported that sedimentation is related to the granularity

of the flour and that the sediment is an agglomeration of the coarse particles rather than swollen protein. He contends that sedimentation is an indication of hardness rather than of the strength of the wheat. According to Zeleny (1960) the sedimentation value is influenced by the quantity and quality of the gluten.

Inheritance of Sedimentation

Long mixing tolerance is important for potential bread wheat varieties. Lebsock et al (1964) examined variation in sedimentation values for parents, F_1, F_3, F_5 and F_6 generations of the spring wheat cross PI 56219-12 x Conley. Sedimentation values were positively correlated with mixing tolerances ($r = 0.61$) and protein content ($r = 0.24$ to 0.52) Heritability values of 56 to 60% were estimated for sedimentation. They concluded that the sedimentation test should be useful for evaluating mixing tolerance of early generation lines from highly variable populations. Zeleny et al (1960) worked with hard red spring wheats representing F_3 generations from a cross between the high protein, strong gluten Conley cultivar and the low protein, weak gluten PI 56219-12 cultivar. All F_3 lines had sedimentation values between those of the two parents. Sunderman et al (1965) examined 66 F_2 and F_3 progeny from the winter wheat cross Itana x Atlas 66 looking at protein content and sedimentation values. Heritability estaimtes for sedimentation were 44% to 64%. They concluded that selection of the 34 F_2 plants with high sedimentation values would have resulted in retention of 90% of the F_3 lines. They found that F_2 protein was positively correlated with F_2 sedimentation ($r = 0.38$), F_2 sedimentation was correlated at the highly

significant level with F_3 sedimentation values, and F_3 protein content was positively correlated with F_3 values.

III. MATERIALS AND METHODS

Two phenotypically diverse wheat cultivars (Yamhill and Inia 66) were hybridized to study the inheritance of protein, lysine, kernel hardness and sedimentation (gluten strength). Yamhill is a low protein, high yielding winter cultivar developed in the Pacific Northwest. It is mid-tall with medium length soft white kernels and mid-season in its maturity with good pastry milling and baking quality. Inia 66 is a high protein, high yielding, hard cultivar developed in Mexico. It is light insensitive, semi-dwarf and very early in its maturity with strong gluten strength and excellent bread milling and baking qualities.

Crosses were made using Yamhill, the winter parent, as the female. A portion of the F_1's were backcrossed to the respective winter and spring parent. The F_2 generation resulted from selfing F_1 plants. In the fall of 1977 parental lines plus F_1, F_2 and BC generations were grown at Hyslop Agronomy Farm in Corvallis, Oregon.

Individual plots of the parents and F_1's consisted of one row 2m long with ten seeds planted per row. The F_2 seed was planted in six rows with ten seeds planted per row. Backcross populations were planted in four rows with ten seeds planted per row. Plants were spaced 20cm apart within the rows and there were 30cm between rows. A randomized block design with four replications was utilized. Each replication consisted of ten plants of each parent, ten plants of the F_1's, 60 plants of the F_2's and 40 plants for each backcross.

Soil type at Hyslop Agronomy Farm is a Woodburn silt loam. At the time of planting, 300kg per hectare of 16-20-0 fertilizer was

incorporated into the seed bed. In addition, 400kg per hectare of nitrogen as urea was applied as a top dressing in the spring. The average rainfall in Corvallis during the 1977-78 growing season was 1150mm. Rainfall on a per month basis for this time period is reported in Appendix Table 1. To avoid possible phytotoxicity, no herbicides were applied and weeding was done by hand.

Six gram samples per plant were used to determine protein, lysine, and kernel hardness values. Four gram samples per plant were taken for sedimentation tests.

Protein

Samples were analyzed by two methods. The first was with the near-infrared reflectance analyzer. Samples were ground on a UDY Cyclone grinder, sieve size 1.0mm. The samples (whole wheat) were placed into the infra-alyzer where a protein value was determined. Samples of known protein content were run every tenth sample during the analysis to ensure reliable results. In the second method protein was measured by Micro-kjeldahl nitrogen analysis. In wheat flour the relationship between the amount of nitrogen and the protein content is expressed by multiplying the nitrogen content of the flour by 5.7. The results obtained by this method corresponded well with the infrared technique. The Micro-kjeldahl procedure is outlined in Appendix Table 2.

Lysine

Samples were analyzed by the near-infrared reflectance analyzer. The analyzer was calibrated with the use of as wide a range of lysine values as possible. The readings for lysine were obtained at the

time as the protein readings.

Hardness

Kernel hardness was measured with the use of the near-infrared reflectance analyzer as outlined by Bruinsma and Rubenthaler (1979). To calibrate the machine, a hardness index of 0 to 100 was designed and 100 samples of all known classes and types of wheat were run. All soft wheats had a hardness index of 0 to 35 while the harder wheats had hardness indices of 55 to 80. Readings for hardness were obtained at the same time as the protein and lysine. Every tenth sample was a wheat sample of known hardness to ensure accuracy.

Sedimentation

Sedimentation was measured using the modified micro-sedimentation technique outlined by Kitterman and Barmore (1969). The sedimentation test as outlined by Zeleny (1947) and modified by Kitterman and Barmore is run with flour ground on mills requiring a minimum seed sample size of 75gms. However, thesis sample size was often limited to 25gms per sample. There are a number of mills which will grind small samples, however, their flour products had not been tested to see if they could be used for sedimentation studies.

Therefore, a preliminary study was set up to test a number of small samples prepared by various methods for sedimentation tests. Sample preparation is outlined in Appendix Table 3. From this study it was concluded that ground wheat flour and whole ground wheat were unacceptable. Micromill flour gave good results for soft wheats but was unacceptable for hard wheats. Based on sedimentation values and flour recovery, tempered micromill flour was determined to be acceptable

for modified micro-sedimentation tests. As a result of the study, four gram wheat samples were tempered to a constant moisture of 12%. The samples were milled on the micromill and sifted for one minute. Throughout the sedimentation trials standard samples of known sedimentation values were run every fifteenth sample to ensure accuracy. The modified micro-sedimentation procedure is outlined in Appendix Table 4.

In each of the replications there were a number of plants which were not harvested, so to evaluate possible differences for the traits measured on an individual plant basis an unequal analysis of variance was used. The F test was utilized to determine if significant differences existed among the generations and within generations including the parental lines. A one way analysis of variance for each generation tested for differences between replications. Mean values for protein, lysine, kernel hardness and sedimentation for parents, F_1's, F_2's and BC generations were compared using the Duncan multiple range test (Steel and Torrie, 1960). Frequency distributions for the parents, F_2 and BC generations were prepared. Where necessary, replication effects were removed and mean square values were used as the generation variance for each character. Narrow sense heritability estimates were calculated using the formula given by Warner (1952). Standard error of the heritability estimate was computed after Ketata et al (1976).

$$h^2_{n.s.} = \frac{V_{F_2} - \frac{V_{BC_1} + V_{BC_2}}{2}}{V_{F_2}}$$

Where V_{F_2} = Variance of the F_2 population

V_{P_1} = Variance of parent 1

V_{P_2} = Variance of parent 2

V_{BC_1} = Variance of the backcross to parent 1

V_{BC_2} = Variance of the backcross to parent 2

$$\text{se.e } h^2_{n.s} = ((2/V_{F_2}{}^2)\ ((V_{B_1} + V_{B_2})^2/df\ F_2 + V_{B_1}{}^2/df\ B_1 + V_{B_2}{}^2/df\ B_2))^{1/2}$$

Where df F_2, df B_1 and df B_2 are the degrees of freedom for the F_2 and backcross generations respectively.

IV. EXPERIMENTAL RESULTS

Due to a large number of missing plants per replication, an unequal analysis of variance was performed. Observations made on an individual plant basis indicated that significant differences were present for the four characters measured (Table 1). No significant differences were found between replications for any of the four characters. Significant generation x replication interactions were noted for protein and lysine.

The coefficients of variation (CV) were high for hardness and sedimentation 31.6 and 27.2% respectively. The CV value for protein was 11.61% while the value for lysine was low at 6.48%.

The mean values of the six populations were analyzed on a plot mean basis for the four traits studied and are presented in Table 2.

The difference in protein levels for the parental varieties was small but significant. Inia 66, which is regarded as a moderately high protein cultivar, had a mean protein value of 11.27%. Yamhill, the low protein parent, had a mean protein value of 10.48%. The F_1 mean of 9.47% was significantly lower than the low protein parent. The F_2 mean fell between the two parents but was not significantly different from either parent. The mean of the backcross to Inia 66 was also between the two parents but not significantly different from either while the backcross to Yamhill gave a mean significantly lower than the low protein parent.

The difference in mean lysine content for the parental varieties was only 0.20% but this difference was significant. Yamhill, with a

Table 1. Summary of the observed mean squares from unequal analysis of variance for protein content, lysine content, kernel hardness and sedimentation involving parents, F_1's, F_2's, BC_I and BC_Y generations.

Source of variation	df	Observed mean squares			
		Protein	Lysine	Hardness	Sedimentation
Generations	5	22.938**	.493**	3239.991**	62.980**
Replication	3	1.009	.038	65.991	3.564
Gen X Reps	15	2.702*	.095*	349.236	2.348
Residual	433	1.481	.047	250.392	1.617
Total	456	1.749	.053	289.673	2.887
Coefficient of Variation %		11.61	6.48	31.56	27.17

*Significant at the 5% level.

**Significant at the 1% level.

mean lysine content of 3.46% falls at the upper end of the lysine values normally reported in wheat. The F_1 mean approached the high lysine parent while the F_2 mean was very similar to the mean value for Inia 66, the low lysine parent. The mean of the backcross to Inia 66 and the mean of the backcross to Yamhill approached the Yamhill lysine mean.

There were significant differences found between the two parents for kernel hardness. Inia 66 is regarded as a cultivar with hard kernels while Yamhill is a soft kernel cultivar. These characterizations are supported by the values obtained in this study. The mean value for hardness for Inia 66 was 60.58% and the mean value for Yamhill was 37.16%. The F_1 and F_2 means were between the two parents. The mean of the backcross to Inia 66 approached Inia while the mean of the backcross to Yamhill was significantly higher than the Yamhill kernel hardness mean.

Significant differences were found between the two parents for sedimentation value (gluten strength). F_1 and F_2 population means were between the two parents with the F_2 mean greater than the F_1 mean. The backcross to Inia 66 gave a population mean higher than the low sedimentation parent but not as high as the high parent. The mean value for the backcross population involving Yamhill was similar to the mean of Yamhill.

To gain additional information regarding the four traits measured, frequency distributions were developed and compared for the five populations.

Protein

A large environmental affect was noted for protein (Table 2). The

Table 2. Mean values for protein, lysine, kernel hardness and sedimentation for the six populations evaluated.

	Mean Values			
	Protein (%)	Lysine (%)	Hardness (%)	Sedimentation (cm)
Yamhill	10.48[a*]	3.46[a]	37.16[a]	2.88[a]
Inia 66	11.27[b]	3.26[b]	60.58[b]	7.32[b]
F_1	9.74[c]	3.39[acd]	50.88[c]	4.20[c]
F_2	10.78[ab]	3.28[bc]	51.55[c]	4.82[c]
BC_I	10.85[ab]	3.24[b]	57.08[bc]	5.62[d]
BC_Y	9.77[c]	3.43[ac]	43.61[d]	3.24[a]

*Ranking of the mean values using Duncan Multiple Range Test.

Treatments with the same letter are not significantly different at the 5% level.

distribution of the parental varieties overlapped one another making genetic interpretation difficult (Fig. 1). The mean value of the F_1 population was lower than the means of the parents and again environmental influence made accurate evaluation of the nature of inheritance difficult. Transgressive segregation was noted for low and high protein content in the F_2 generation. Backcross distributions were skewed toward the distribution of the recurrent parent.

Lysine

Although the lysine content for the parental varieties was significantly different, variation for lysine appears to be limited. The F_2 distribution (Fig. 4) shows that it would be possible to recover progeny with lysine values equal in magnitude to the high lysine parent, Yamhill. Backcross to Inia 66 shifted the population toward the Inia 66 parent and a backcross to Yamhill skewed the distribution toward Yamhill. No transgressive segregation for high lysine content was observed.

Hardness

Parental distributions for hardness show two fairly distinct classes (Fig. 6). The F_1 mean value was between the two parents. The F_2 distribution spanned both the high and low parental distributions. Transgressive segregation was noted for both soft and hard kernel types. One backcross to either parent produced a preponderance of progeny approaching the recurrent parent.

Sedimentation

The parental cultivars formed two distinct groups for sedimentation. The F_2 distribution (Fig. 10) was skewed toward the distribution of

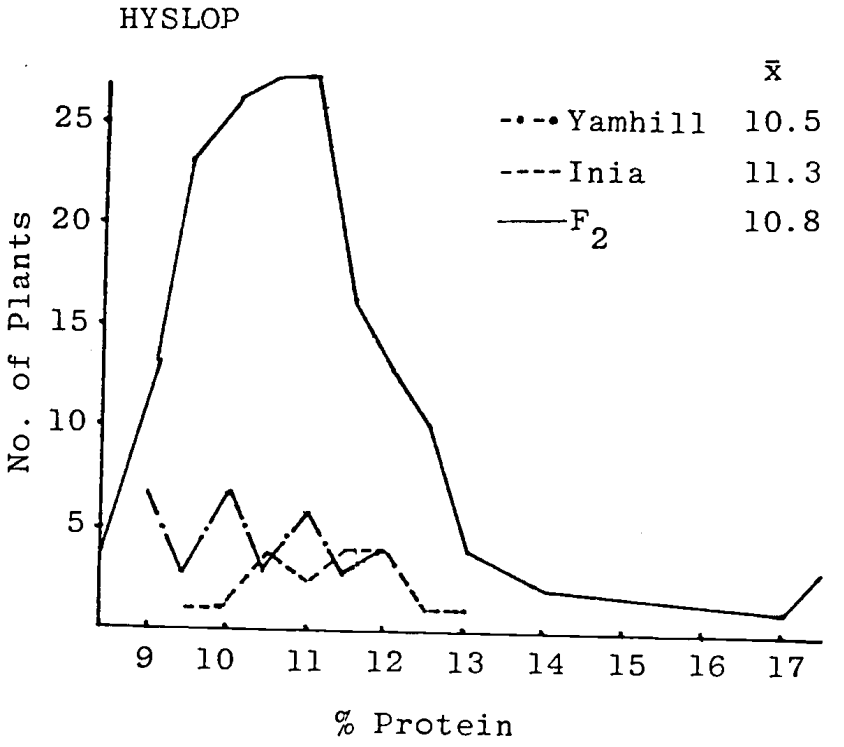

Fig. 1 Frequency distribution of parents and F_2 plants for percent protein.

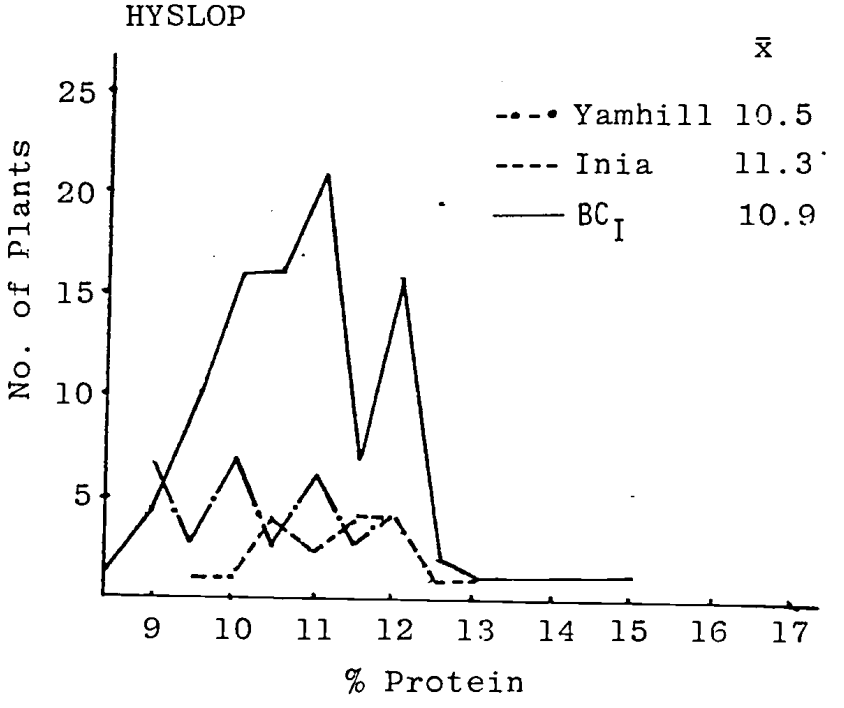

Fig 2 Frequency distribution of parents and the backcross to Inia 66 for percent protein.

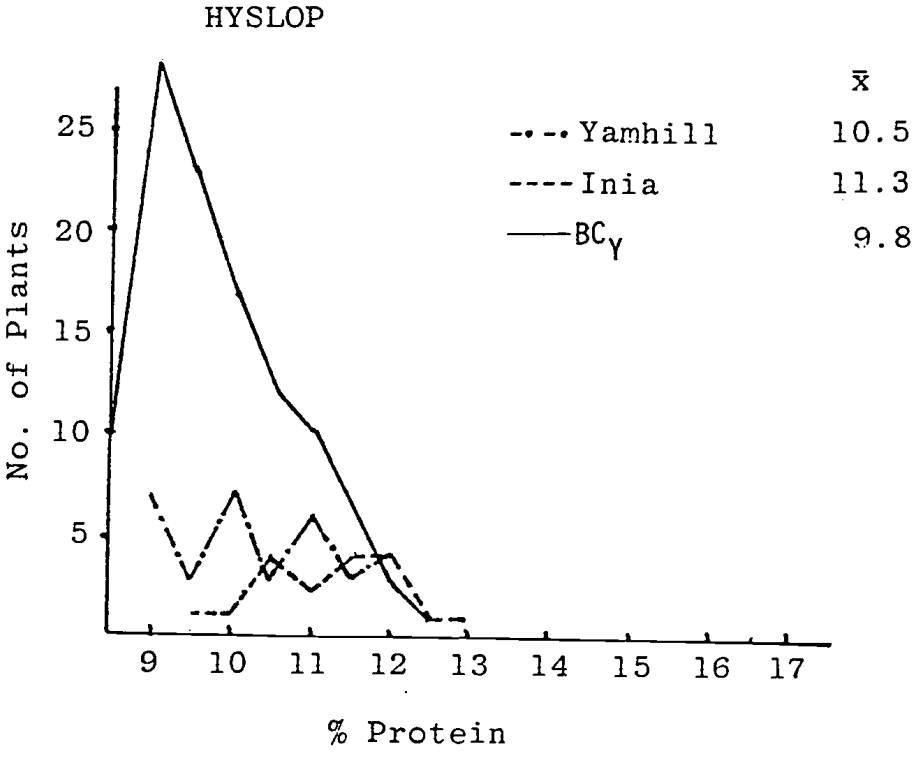

Fig. 3 Frequency distribution of parents and the backcross to Yamhill for percent protein

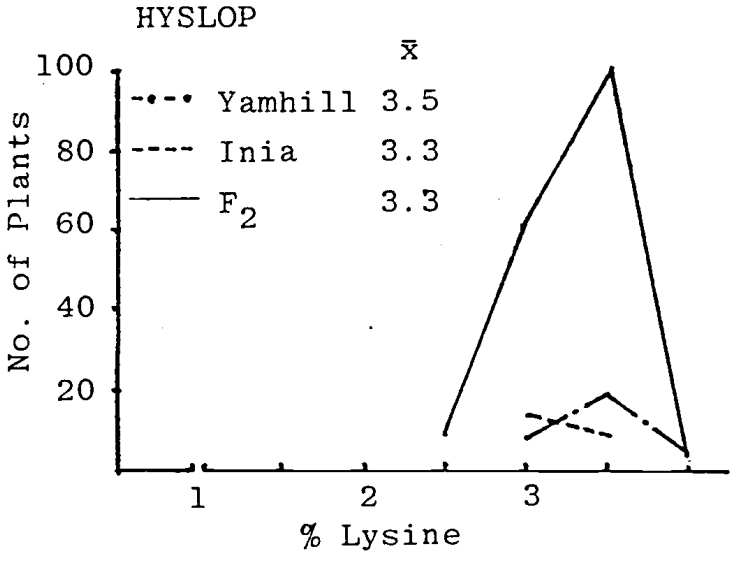

Fig. 4 Frequency distribution of parents and F_2 plants for percent lysine.

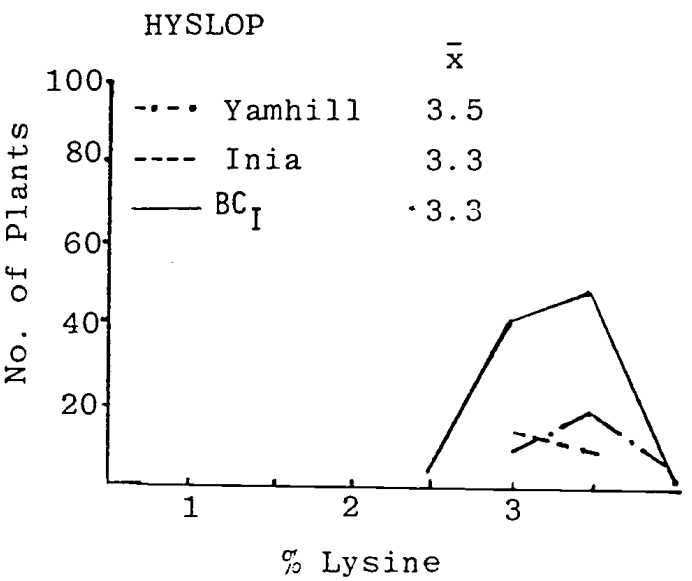

Fig. 5 Frequency distribution of parents and the backcross to Inia 66 for percent lysine.

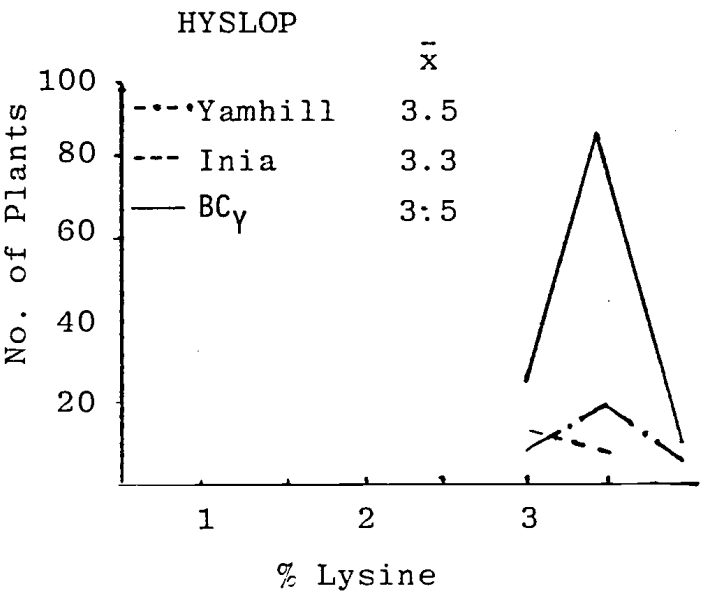

Fig. 6 Frequency distribution of parents and the backcross to Yamhill for percent lysine.

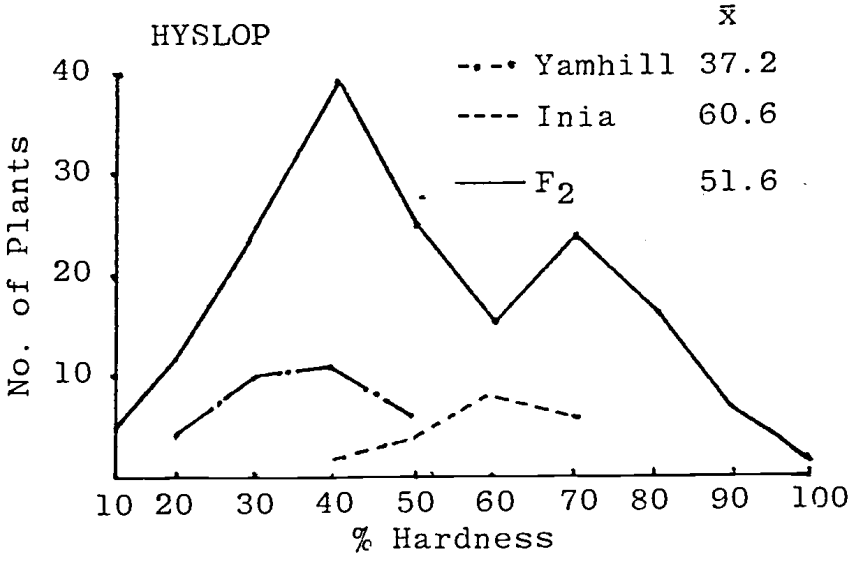

Fig. 7 Frequency distribution of parents and F_2 plants for percent hardness.

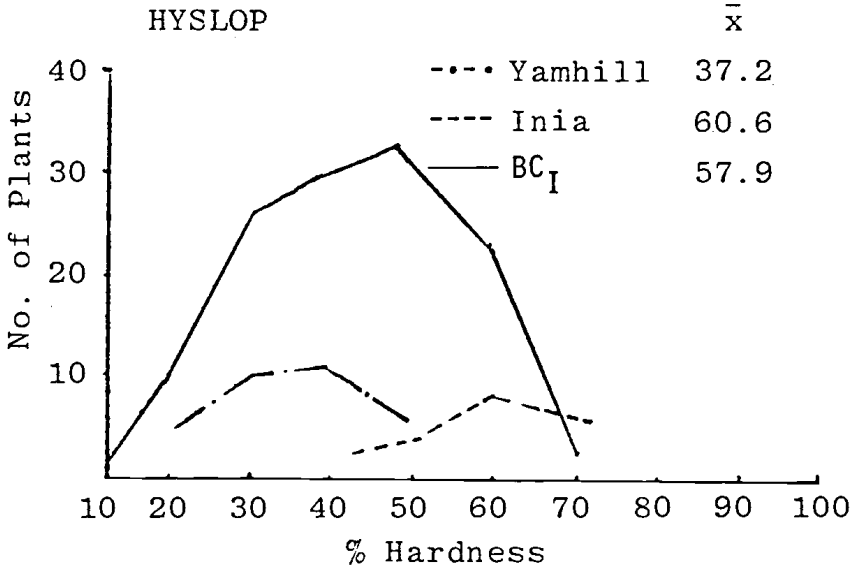

Fig. 8 Frequency distribution for parents and the backcross to Inia 66 for percent hardness.

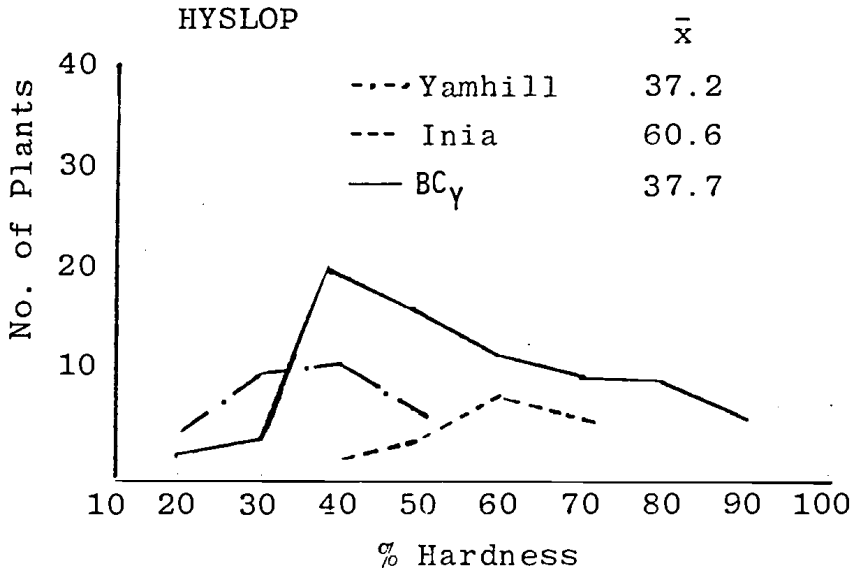

Fig. 9 Frequency distribution for parents and the backcross to Yamhill for percent hardness.

the low parent and no progeny were recovered which approached the high parent. One backcross to the low sedimentation parent, Yamhill, gave a majority of individuals similar in sedimentation value to Yamhill. One backcross to Inia 66 moved the progeny distribution toward Inia 66 but no individuals were recovered with sedimentation values as high.

To determine the proportion of the total variance which was due to additive gene action, narrow sense heritability estimates were determined and are shown in Table 3 for the four traits measured. The estimates for all four traits were high with protein and kernel hardness being 1.10 ± 0.14 and 0.90 ± 0.17 respectively. The narrow sense heritability estimates for lysine and sedimentation were 0.69 ± 0.23 and 0.58 ± 0.21 respectively.

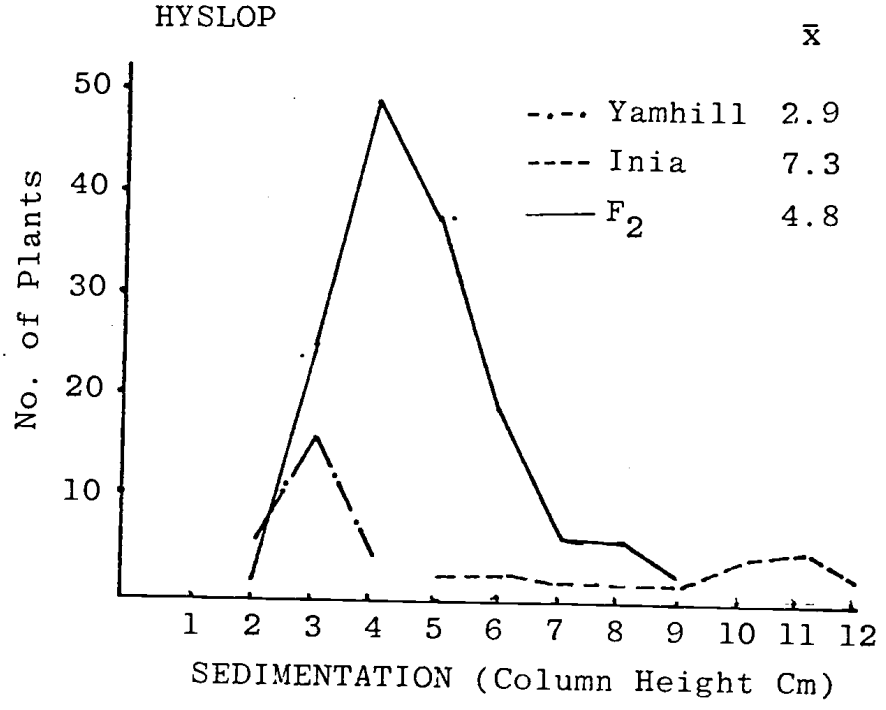

Fig. 10 Frequency distribution for parents and F_2 plants for sedimentation (cm).

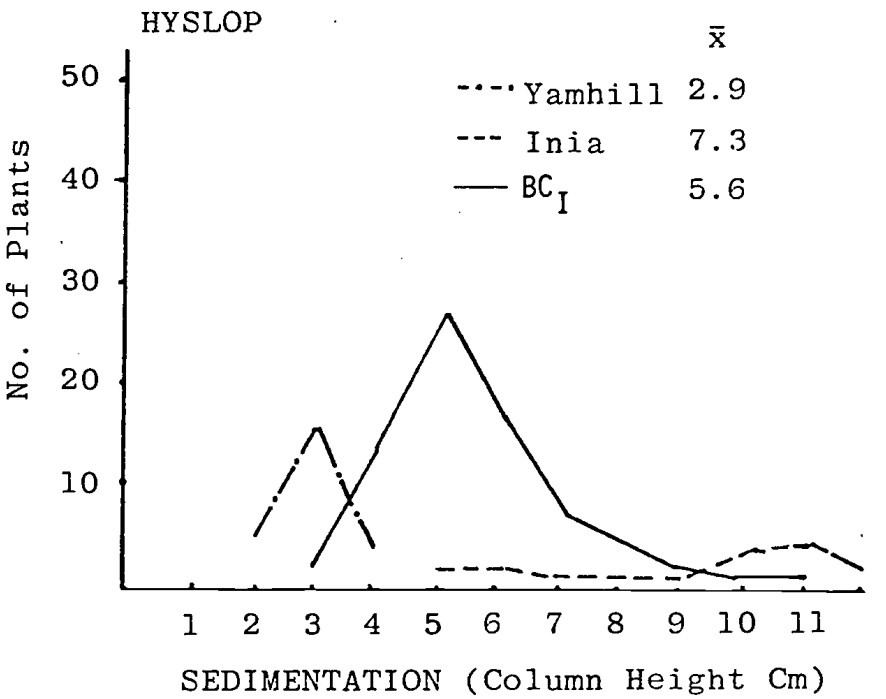

Fig. 11 Frequency distribution for parents and the backcross to Inia 66 for sedimentation (cm).

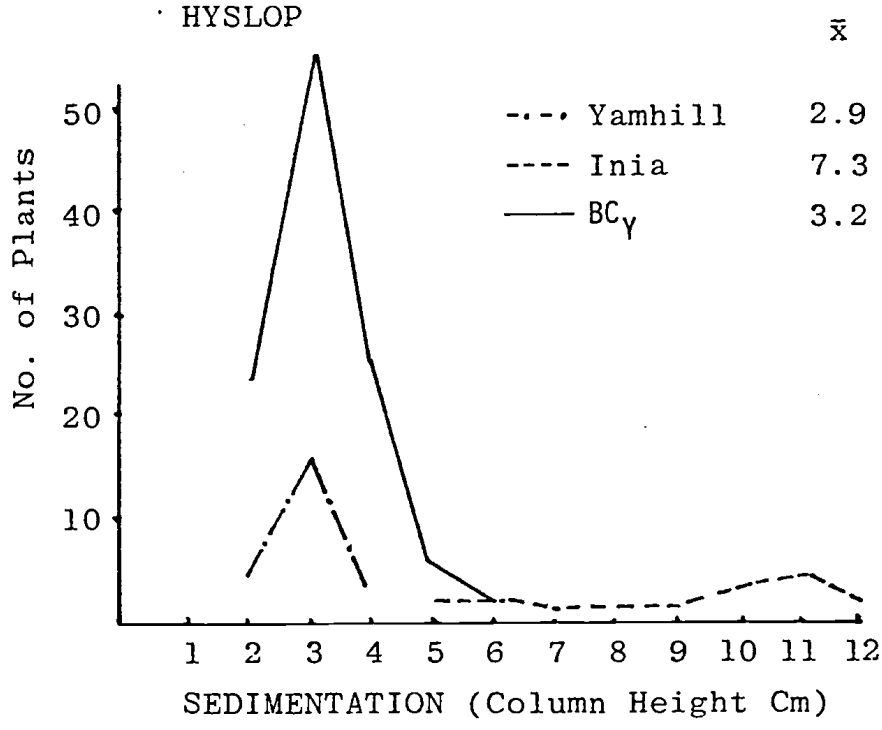

Fig. 12 Frequency distribution for parents and the backcross to Yamhill for sedimentation (cm).

Table 3. Narrow sense heritability estimates with standard errors for the four traits measured.

Trait	$h^2_{n.s.}$
Protein	1.10 ± 0.14
Lysine	0.69 ± 0.23
Hardness	0.90 ± 0.17
Sedimentation	0.58 ± 0.21

V. DISCUSSION

Wheat will continue to be an important source of nourishment for an ever increasing world population. Therefore, breeders will not only have to increase yield but improve the nutritional quality as well. Quality improvement will involve increasing protein content and improving the balance of limiting essential amino acids such as lysine. At the same time it is essential to ensure that the wheat flour will produce end products acceptable to consumer tastes and demands. Of concern in this area are desired gluten strength and kernel hardness.

Past attempts to meet the challenges of increasing world demand for food have been focused on hybridizations within winter or spring cultivars. The combination of the emphasis on crossing within winter or spring types and the fact that these types are grown under differing environmental conditions has led to a divergence in the genetic makeup of the two types. Current attempts to increase the genetic diversity for future improvement include programs of systematically crossing winter and spring cultivars. While creating additional genetic diversity, concern has been expressed that higher yields may be achieved at the expense of improved quality. To answer these concerns, the breeder must first evaluate how much genetic diversity exists in the population for the characteristics of concern. Once the variability has been assessed, the breeder must determine if the variability is easily transmitted to the offspring and to what extent the traits are influenced in their expression by the environment.

The two parental cultivars used in this study, which represented

winter and spring types, differed in protein content by 1.79% and this difference was significant. Due to the growing conditions at Hyslop Agronomy Farm, the full genetic potential for protein content of Inia 66 was not realized. Inia 66 is a spring wheat which was planted in the fall for this study, and this may have been in part responsible for the low protein expression. Conversely, the protein level observed for Yamhill was elevated above normal values for this site. When the mean of the F_1 population was examined, it was below the lowest parent while the F_2 mean was intermediate to the two parents. This would suggest that the environment played a major role in influencing protein content as supported by the large coefficient of variation calculated for protein content. Backcrosses to either parent shifted the population means toward the recurrent parent and in fact the backcross to Yamhill shifted the mean below the Yamhill parent. The narrow sense heritability estimate for protein was high (1.10 ± 0.14). Values greater than one can be realized due to sampling error and this appears to be the case for protein content in this study. The heritability estimates for the other traits may also reflect similar biases in a positive or negative direction. The high heritability (narrow sense), the ease with which parental types were recovered in the backcross generations, and the intermediate position of the F_2 mean all suggest that genetic variance associated with protein was largely additive in nature and a few major genes may be controlling protein content in this cross.

Perhaps of even greater importance to the plant breeder is the transgressive segregation observed in the F_2 population. The range of protein in the F_2 generation was from 8.5 to 17.6%. It would appear

that with the additive nature of the genetic variance and the transgressive segregation observed that effective selection for high and low protein could be made within the cross.

Although the difference in lysine content of the parental cultivars was very small (0.20%), it was significant. Yamhill had a mean lysine value approaching the upper limit previously reported for wheat. The mean of the F_1 population was close to the midparent value while the F_2 was also between the two parents but lower than the F_1 mean. Interpretation of the type of gene action involved is difficult but it appears that a small number of genes may be involved which behave in an additive manner. Narrow sense heritability estimates were intermediate for lysine at 0.69 ± 0.23. There was no transgressive segregation observed for high lysine in the F_2. The backcross to Yamhill shifted the population toward the Yamhill mean but no individuals were found which exceeded Yamhill. This information would indicate that although crossing diverse genotypes will not necessarily result in a loss of lysine content, it would be difficult to select for individuals with higher lysine than Yamhill and further improvement for lysine content is doubtful.

Significant differences were found between the two parents for kernel hardness. F_1 and F_2 means were intermediate to the two parents suggesting the genes were additive in their action. Based on the F_2 distribution it appears that soft kernel texture is dominant to hard and that the parents differed by only a few alleles. Backcrosses to either parent shifted the populations toward the recurrent parent. Transgressive segregation in the F_2 generation was found for both soft and hard kernel types. This fact, along with the high narrow sense

heritability estimate (0.90 ± 0.17) would suggest genes with additive effects account for much of the genetic variance found for kernel texture and that selection for hard or soft types would be effective.

Dominant gene action appears to account for much of the variation found for sedimentation (gluten strength). The parents differed significantly in sedimentation values and the F_1 and F_2 means were below the midparent value. The F_2 distribution was skewed toward the low sedimentation parent, Yamhill. One backcross to the high sedimentation parent, Inia 66, shifted the population toward higher sedimentation values (stronger glutens) but no individuals were recovered which approached the Inia 66 parent. One backcross to Yamhill shifted the population back to the Yamhill value. No transgressive segregation was observed for low or high sedimentation. The narrow sense heritability estimate for sedimentation was high (0.58 ± 0.21). It would appear that low sedimentation is dominant to high and that it may be difficult to recover high sedimentation types. At the same time, further improvement for low sedimentaion appears limited.

From this study several observations can be made which are useful to the plant breeder. For protein, the transgressive segregation observed in the F_2 generation would suggest that effective selection can be made for high or low protein. Yamhill was shown to be a relatively high lysine parent and when used in a crossing program high lysine could be retained; however, little or no opportunity exists in this cross to find segregates higher in lysine than Yamhill. Kernel hardness appears to be influenced by a few genes which reflect an additive type of action. Selection should be effective for both soft and hard types.

Low sedimentation appears to be dominant to high and breeders interested in bread wheats (high sedimentation or strong gluten wheats) must exercise caution in crossing low and high sedimentation types.

Of particular significance to the cereal breeder is that winter x spring crosses do not potentially limit quality and the approach appears to be a promising method for creating additional genetic diversity if high yield and high protein can be combined.

VI. SUMMARY AND CONCLUSIONS

To study levels and inheritance of protein, lysine, kernel hardness and sedimentation (gluten strength) high protein cultivar Inia 66 and low protein cultivar Yamhill were hybridized. A portion of the F_1's generated were then backcrossed to both parents. Parental lines, F_1, F_2 and backcross populations were grown at Hyslop Agronomy Farm in Corvallis, Oregon.

Protein content was analyzed by the use of near-infrared reflectance and micro-kjeldahl protein analysis. Hardness was measured with the use of hardness index derived from near-infrared analysis. Lysine was measured with the near-infrared reflectance analyzer which was calibrated using amino acid analysis. Sedimentation values were obtained by modified micro-sedimentation procedures.

To evaluate if significant differences existed for the traits measured, an unequal analysis of variance on a per plant basis was used. Duncan's multiple range test was used to compare population mean values. Frequency distributions were prepared for each generation. Narrow sense heritability estimates were calculated using the variances of the parents, F_2 and backcross generations.

From this study the following conclusions were made:

1. Protein content was greatly influenced by the environment as was evidenced by the mean value of the F_1 in relation to the F_2 and parental populations and the high CV value. The mean value of the F_2, the high narrow sense heritability estimate and ease of recovering parental types in the backcross

generations all suggest that genes influencing protein content are largely additive in their action. Transgressive segregation was found in the F_2 generation for both high and low protein individuals and selection appears to be possible for either type.

2. Yamhill had a mean lysine value which approaches the upper limit previously reported for wheat. F_1 and F_2 mean values, frequency distributions and the high narrow sense heritability estimate for lysine suggest that relatively few major genes are involved and that much of the genetic variance is additive. Yamhill may be used as a high lysine source and although increases in lysine above Yamhill do not appear possible, levels of lysine equal to the high lysine parent are maintainable when Yamhill is used in a crossing program.
3. From the intermediate F_1 and F_2 mean values, the F_2 distribution and the high narrow sense heritability estimate, kernel hardness appears to be under the control of genes which behave in an additive manner. The parents appear to differ by only a small number of genes. Trangressive segregation in the F_2 generation for both kernel hardness and softness suggest that substantial progress from selection would be possible in either direction.
4. Significant differences were observed between the two parents for sedimentation value. F_1 and F_2 mean values indicate that the genes reflect mainly non-additive action for gluten strength. No transgressive segregation was observed in the

F_2 for either low or high sedimentation. Low sedimentation appears to be dominant from the frequency distributions prepared.

5. Crossing winter x spring genotypes does not appear to produce adverse effects for the traits measured in this study. A single backcross to either parent shifted the distribution back to the recurrent parent for protein, lysine and kernel hardness. Winter x spring crosses offer a potentially important method for creating additional genetic variability for both agronomic and quality factors in wheat.

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APPENDIX

Appendix Table 1. Precipitation on a per month basis for Corvallis, Oregon for the 1977-78 growing season.

Month	Precipitation (mm)
October	65.5
November	206.0
December	280.2
January	186.4
February	108.7
March	54.6
April	125.5
May	91.7
June	23.9
July	7.4

Appendix Table 2. Procedure for Micro-kjeldahl nitrogen analysis.

Reagents

1. 4.0 gm Kelpack (se catalyst) consisting of K_2SO_4, $Na_2S_2O_3 \cdot 5H_2O$, $CuSO_4$ and selenium.
2. 12.0 ml H_2SO_4.
3. 50.0 ml H_2O.
4. 55.0 ml 10 M NaOH.
5. 4% Boric acid indicator solution containing methyl red and bromocresol green indicator.
6. 0.1253 N HCl.

Procedure

1. A 1.00 gm sample of whole wheat flour is degested with 12.0 ml of H_2SO_4 and 4.0 gm of Kelpack for 22 minutes at 410° C.
2. The material is cooled for seven minutes.
3. Fifty mls of H_2O are added and the mixture is transferred to the distillation unit.
4. Fifty-five mls of 10 M NaOH are added and steam is bubbled through the solution giving ammonia.
5. The ammonia is distilled into a boric acid indicator solution.
6. The solution is titrated with 0.1253 N HCL to the color change point (back to purple).
7. The amount of nitrogen in the sample is calculated from the amount of acid required to neutralize the ammonia.

Appendix Table 3. Micro-sedimentation preliminary study preparation.

Sample #	Preparation	Product
1	Buhler Mill	Flour
2	UDY Cyclone Mill (0.5 mm screen)	Whole Ground Wheat
3	UDY Cyclone Mill (0.5 mm screen) sifted on the micromill	Ground Wheat Flour
4	Micromill	Untempered Micromill Flour
5	Tempered and Micromilled	Tempered Micromill Flour

Appendix Table 4. Procedure for modified micro-sedimentation.

Reagents

1. 100% isopropyl alcohol.
2. H_2O containing 4.0 mg bromophenol blue per liter.
3. Lactic acid stock solution. This is prepared by diluting 250 ml of 85% lactic acid to 1 liter with H_2O. The solution is refluxed for 6 hours.
4. 180 ml of the lactic acid stock solution, 200 ml of isopropyl alcohol and water are mixed to make 1 liter.

Procedure

1. 0.40 gm of flour is weighed into 10 ml cylinders.
2. The cylinders are held in a horizontal position and tapped on the bench top a few times to jar the flour into a wedge shape. The cylinders are then placed in a rack which holds them at a 30° angle.
3. A 20 second interval between samples permits 15 samples to be tested at a time. The timing is started and simulataneously 6 ml of hydration water is added. The cylinder is stoppered and mixed on the cyclomixer for 7 to 8 seconds to disperse the flour. About 13 seconds have elapsed. The cylinder is unstoppered, 3.0 ml of lactic acid reagent is added, the cylinder is stoppered and placed in a rocker mixer. Each tube is treated in this manner one every 20 seconds.
4. The cylinders are removed one at a time from the rocker mixer after each has mixed for five minutes. The tubes are placed in the cups of a centrifuge and centrifuged for two minutes, at a maximum speed of 850 (130-140 r.c.f.).
5. The cylinders are removed and unstoppered. The supernatant is removed with suction and the cylinders are replaced in a rack.
6. Up to 7 1/2 ml of volume adjusting solution is added to each tube at 20 second intervals and each is placed on the rocker mixer after a thorough hand shaking.
7. After 5 minutes the cylinders are removed at 20 second intervals and placed upright in a rack.
8. When all cylinders have been removed and are standing in the rack, they are left to stand for 10 minutes.
9. At the end of this time the height of sediment is measured to the nearest 0.1 centimeter.