Grain protein and grain yield as functions of dry matter, plant protein, and chlorophyll characteristics in elite international winter wheats
by Mohamed Ali Al-Khawlani

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science i n Agronomy
Montana State University

Abstract:
Wheat grain protein content is important for product utilization and nutritional qualities. Improving protein content is difficult because of the inverse relationship with grain yield. The relationship of dry matter, plant protein and chlorophyll characteristics to grain pfotein and grain yield was studied in 66 international winter wheats (Triticum aestivum L.). Field experiments include low, medium, and high N fertility regimes in 1984 and 1985.

The cultIvars differed for all measured traits. A significant but low negative correlation between grain yield and percent grain protein was found only in 1984. This suggested simultaneous increases in grain yield and grain protein could be achieved by selection. Total plant protein was positively correlated with biological yield and grain yield, but not correlated with percent grain protein.

Nitrogen harvest index (NHI) decreased with increasing soil N levels. High grain protein cultivars were more efficient than medium and low grain protein cultivars at any soil N level above 110 kg/ha. A positive correlation between nitrogen harvest index (NHl) and percent grain protein was found in 1985.

Chlorophyll concentration during grain filling period was correlated with grain yield and total plant protein. High grain protein cultivars had longer chlorophyll duration after anthesis than low protein cultivars.

Nitrogen harvest index, total plant protein, and chlorophyll duration after anthesis accounted for 88% and 94% in 1984, and 94% and 90% in 1985, of the total variation among cultivars in grain yield and percent grain protein, respectively. Simultaneous increases in grain yield and percent grain protein could be achieved by selecting for high total plant protein, high N-translocation efficiency, and longer chlorophyll duration after anthesis in wheat.

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in
Agronomy

MONTANA STATE UNIVERSITY
Bozeman, Montana

May 1986

APPROVAL

of a thesis submitted by

Mohamed Ali Al-Khawlani

This thesis has been read by each member of the author's graduate committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ACKNOWLEDGMENTS

I wish to express my sincere appreciation to the following:

Dr. G. Allan Taylor, my major advisor, for his advice, friendship, and encouragement through the course of this study.

Dr. Jarvis H. Brown, Dr. C. F. McGuire, and Dr. John M. Martin for sharing their time, their advice, and their enthusiasm while serving on my committee.

The World Bank and Food and Agriculture Organization for financial support that made this program successful.

My wife, Fathia, and son, Nabel, for their patience and love.

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ABSTRACT

Wheat grain protein content is important for product utilization and nutritional qualities. Improving protein content is difficult because of the inverse relationship with grain yield. The relationship of dry matter, plant protein and chlorophyll characteristics to grain protein and grain yield was studied in 66 international winter wheats (Triticum aestivum L.). Field experiments include low, medium, and high N fertility regimes in 1984 and 1985.

The cultivars differed for all measured traits. A significant but low negative correlation between grain yield and percent grain protein was found only in 1984. This suggested simultaneous increases in grain yield and grain protein could be achieved by selection. Total plant protein was positively correlated with biological yield and grain yield, but not correlated with percent grain protein.

Nitrogen harvest index (NHI) decreased with increasing soil N levels. High grain protein cultivars were more efficient than medium and low grain protein cultivars at any soil N level above 110 kg/ha. A positive correlation between nitrogen harvest index (NHI) and percent grain protein was found in 1985.

Chlorophyll concentration during grain filling period was correlated with grain yield and total plant protein. High grain protein cultivars had longer chlorophyll duration after anthesis than low protein cultivars.

Nitrogen harvest index, total plant protein, and chlorophyll duration after anthesis accounted for 88% and 94% in 1984, and 94% and 90% in 1985, of the total variation among cultivars in grain yield and percent grain protein, respectively. Simultaneous increases in grain yield and percent grain protein could be achieved by selecting for high total plant protein, high N-translocation efficiency, and longer chlorophyll duration after anthesis in wheat.

INTRODUCTION

Wheat grains constitute the stable food of a large proportion of the world population. Therefore, wheat protein represents a major source of protein for both humans and animals. Grain protein is an important factor for both baking and nutritional properties of bread wheat. Increasing grain yield and grain protein simultaneously is the ultimate goal for many wheat breeding programs around the world. The simultaneous improvement of grain yield and grain protein is difficult because of their inverse relationship. However, cultivars with high grain yield and high percent grain protein have been obtained (Johnson et al., 1967). This suggests improving both grain yield and protein content by selection is possible. Increased grain yield and percent grain protein may be associated with increased use of nitrogenous fertilizers or increased efficiency in translocating nitrogenous compounds from vegetative parts of the plant to the grain. Higher costs of nitrogenous fertilizers in recent years have drawn attention to the creation of genotypes with an improved efficiency of nitrogen utilization (Austin et al., 1977).

This study was conducted to examine the genetic variation and the relationships of characters related to grain yield and percent grain protein in elite international winter wheats.

LITERATURE REVIEW

Nitrogen and Dry Matter

Numerous investigators have reported significant inverse relationships between grain yield and percent grain protein in spring and winter wheats (Terman et al., 1969; Halloran, 1981; Bhatia, 1975; Loffler and Busch, 1982). The range of correlation coefficients from -0.48 to -0.58 suggests no genetic limitations for improving both grain yield and grain protein percentage in wheat. Stuber et al. (1962) reported high-yielding lines with high grain protein content were found in an F2 popution of a high x low protein wheat cross. This suggests simultaneous improvement of grain yield and grain protein is possible.

Wheat plants are known to accumulate most of their nitrogen in the vegetative parts prior to anthesis. The nitrogen is translocated to the developing grain after anthesis. McNeal et al. (1968) found the nitrogen content of vegetative parts of seven spring wheat genotypes (leaves, stems, and head chaff) decreased after anthesis, while grain nitrogen content increased. This is indirect evidence for translocation of nitrogenous compounds from vegetative organs to the developing grains. Translocation of labeled amino acids from culms to grains is direct evidence of mass translocation of nitrogen to the grain (Mikesell et al., 1971). Nitrogen translocation efficiency represents the ability of a genotype to translocate nitrogenous compounds from the vegetative parts to the grains. The efficiency of partitioning of nitrogen between straw and grains is expressed as nitrogen partitioning efficiency (Loffler and Busch, 1982), nitrogen translocation efficiency (Halloran and Lee, 1979), or nitrogen harvest index (Desai and Bhatia, 1978). It is calculated as the ratio of grain nitrogen to total plant nitrogen. Significant differences among wheat cultivars were found for nitrogen translocation efficiency (Halloran and Lee, 1979; Dubois and Fossati, 1981; Loffler and Busch, 1982; Loffler et al., 1985). Halloran (1981) found nitrogen translocation efficiency decreased with increasing availability of soil nitrogen, but the high grain protein cultivars remained highly efficient at high soil nitrogen levels. Loffler and Busch (1982) reported selection for high nitrogen harvest index significantly improved grain yield in the progeny of three crosses of hard spring wheat genotypes. Grain protein content significantly increased in one population, with no reductions in the others. Nitrogen translocation efficiency can be an important criterion for improving grain yield (Dubois and Fossati, 1981), or grain protein content (Halloran and Lee, 1979; Loffler et al., 1985).

Total plant nitrogen can be considered as an indicator of the efficiency of nitrogen uptake (Desai and Bhatia, 1978). The physiological basis for high grain yield and high percent grain protein in wheat appeared to be associated with greater nitrogen uptake. Increased total plant nitrogen at maturity or more efficient and complete translocation of nitrogenous compounds from the vegetative plant parts to the grains are likely. Total plant nitrogen at maturity was positively correlated with grain yield, but not significantly correlated with grain protein content (Desai and Bhatia, 1978; Cox et al., 1985b; Loffler et al., 1985). This suggests selection for high total plant nitrogen could improve grain yield without reducing percent grain protein. Johnson et al. (1967) found nitrogen uptake and nitrogen translocation efficiency functions were separate and independent physiological systems in wheat plants. Both total plant nitrogen and nitrogen translocation efficiency could be used in selecting wheat genotypes for efficient nitrogen utilization (Rao et al., 1977).

Studies of characteristics related to nitrogen utilization provide useful information for parent selections and planned crosses. Edwards et al. (1978) crossed two spring wheat genotypes with complementary values of total reduced plant nitrogen and nitrogen translocation efficiency. The high grain protein progeny had a combination of both high total reduced plant nitrogen and high nitrogen translocation efficiency. Bhatia (1975) concluded that grain protein yield per unit ground area provides a good selection criterion for improving protein productivity in spring wheat cultivars. McNeal et al. (1982) also found selection for high grain protein yield increased grain yield and protein productivity in the progeny of spring wheat crosses.

Chlorophyll Content

The negative association between grain yield and percent grain protein is due in part to the competition between carbohydrate and protein accumulation for assimilates and energy in plants (Bhatia and Robson, 1976). Penning de Vries et al. (1974) concluded 1 g of glucose produced by photosynthesis in plants can be used to produce 0.83 g of carbohydrate or 0.40 g of protein (assuming nitrate to be the N source).

Increasing both grain yield and grain protein content could be achieved by increasing photosynthetic output, by increasing the rate of photosynthesis, and by extending the period of photosynthetic activity (Bhatia and Robson, 1976). Cox et al. (1985b) found 10 to 22% of the total plant nitrogen accumulated after anthesis. They concluded cultivars with longer green tissue duration assimilate more nitrogen than cultivars with short green tissue duration. Mikesell et al. (1971) found similar N content in flag leaves of high and low protein wheat lines at anthesis. They reported removing the flag leaves at anthesis had little effect on grain N content of low protein wheat lines, but greatly decreased grain N content of high protein wheat lines. They concluded the viability and longevity of flag leaves are important for high protein lines. High protein lines continued assimilation of N after anthesis. Neales et al. (1963) examined the effect of leaf removal at anthesis on grain N content. They found leaf removal at anthesis reduced the N uptake and the grain N content at maturity. Spiertz et al. (1971) concluded from 61 to 81% of the variation of grain yield could be statistically predicted by green area duration of flag leaf and peduncle. Rahman (1983) found a positive correlation between net photosynthetic rate and chlorophyll content per unit leaf area in couchgrass.

MATERIALS AND METHODS

Cultivars

Sixty-six cultivars selected from four International Winter Wheat Nurseries (1980, 1981, 1983, 1984) were used in this study (Table 1).

Table 1. Names and origins of cultivars used.

Name	Origin	Name	Origin	Name	Origin
Brule	USA,NE	Sutjeska	Yugoslavia	Vratza	Bulgaria
Grana	Poland	Lavrin-32	Romania	Daws	USA,WA
Bezos.1	USSR	Orov.	Yugoslavia	MV-22-27	Hungary
AW12399	E.Germany	F29-75	Romania	Vala	Czechsl.
Arina	Switzer.	MV-7	Hungary	Stephens	USA,OR
NE7060	USA,NE	Horosh.	Japan	Ogosta	Bulgaria
Odissa-4	USSR	CA8055	China	WWP.4258	Austria
Atlas-66	USA,IN	NS-15-89A	Yugoslavia	Vega	Bulgaria
Lavrin-24	Romania	Katya a-1	Bulgaria	NS2630-1	Yugoslavia
Lethb.32	Canada	Feng-Kang	China	Sudova S.	Bulgaria
Martonv.5	Hungary	Saiente	Italy	Loudog.	Bulgaria
Purdue	USA,IN	F29-76	Romania	Bounty	England
Blueboy	USA,NC	GK-Prot.	Hungary	Pai Yu P.	China
Houser	USA,NY	Clement	Netherlands	NS2699	Yugoslavia
Jana	Poland	Doina	Romania	NE79Y90576	USA,NE
Alcedo	E.Germany	WWP.4394	Austria	NSR-1	Yugoslavia
Aura	Finland	Bastion	Netherlands	Adams	Austria
MV-6	Hungary	TX71A562-6	USA,TX	Inernio	Italy
Martonv.	Hungary	WW330	Australia	Trakia	Bulgaria
NS2699	Yugoslavia	Chokwang	Korea	Marisml.	England
Redwin	USA,MT	Centurk	USA,NE	PL V	USA,KA
Lancota	USA,NE	MT 7811	USA,MT	Norwin	USA,MT

The cultivars were selected to provide a wide range of grain protein content, grain yield, and genetic backgrounds. The cultivars originated from 20 countries. They were grouped by height into three groups to reduce interplot competition with 22 cultivars in each group:

75 - 85 cm -- Short

85 - 95 cm -- Medium

>95 cm -- Tall

Experimental Design

The three height groups were planted separately in a completely randomized block field experiment with six replicates per harvest in 1984 and 1985 near Bozeman. The groups were randomized within each nitrogen level. The cultivars were planted in hill plots with 0.3 m spacing. Thirty seeds of each cultivar were planted in each hill plot. Seeds were dropped in 3-4 cm deep holes dug with hoes. Each replicate was surrounded by a short winter wheat cultivar to avoid shading.

Soil Nitrogen

The soil contained 110 and 22 kg/ha NO3-N in one meter depth in 1984 and 1985 harvest years, respectively. The experiments included three soil nitrogen levels in 1984 and 1985. The first experiment had no nitrogen added in both years (N0). The second experiment had 85 and 110 kg/ha nitrogen added as ammonium nitrate (NH_4NO_3) (34-0-0) in 1984 and 1985, respectively (N1). The third experiment had 170 and 220 kg/ha NH_4NO_3 added in 1984 and 1985, respectively (N2). Fertilizer was applied in mid-May. These soil nitrogen levels in two years totaled six soil nitrogen environments.

Chlorophyll Estimations

The need to estimate chlorophyll concentrations in flag leaves of field grown wheat cultivars required a nondestructive method of analysis. Rahman (1983) suggested visual color ratings provide an estimate of chlorophyll content per unit area. He found a positive correlation between visual color ratings and chlorophyll content per unit area.

A chlorophyll meter similar to one used by Wallihan (1973) to estimate chlorophyll concentrations in leaves of citrus trees was used in this study. Calibration of the chlorophyll meter included samples of leaves taken from 17 field grown wheat cultivars. The leaves were selected to provide a wide range of chlorophyll content on the basis of their color (dark green to yellow). Light absorbance readings were taken by chlorophyll meter and chlorophyll concentrations were estimated by extraction (Arnon, 1949).

Plotting chlorophyll concentration (mg/g fresh weight) against absorbance readings showed 91% of the total variation in chlorophyll concentration could be statistically predicted by absorbance readings taken by chlorophyll meter. The chlorophyll meter provided a good estimate of chlorophyll concentration in wheats.

Measurements

Heading dates and plant height were recorded for each hill plot. The heading date was the number of days from January 1 until 50% of the heads in a plot were fully out of the boot. Plant height was measured in centimeters from the soil surface to tip of the majority of spikes within a plot, excluding awns.

Flag leaf chlorophyll concentration of the 66 cultivars was estimated using the chlorophyll meter during grain filling period; four times in 1984 and five times in 1985. Chlorophyll duration was the number of days from anthesis until 75% of flag leaves in a plot turned yellow.

The following data were recorded for each experimental unit:

1. Grain Protein Percentage (GPP) -- amount of protein expressed as percent of the dry weight of the grain.
2. Grain Yield (GY) -- grain weight (g/plot).
3. Biological Yield (BY) -- total dry weight (g/plot) of aerial biomass including grain.
4. Harvest Index (HI) -- GY/BY.
5. Total Plant Protein (TPP) -- amount of protein (g/plot) in the aerial biomass at maturity including grain.
6. Grain Protein Yield (GPY) -- amount of protein (g/plot) in the grain (GY x GPP).
7. Nitrogen Harvest Index (NHI) -- GPY/TPP.

Percent protein in the grain (Williams, 1979) and the straw were estimated using the Near Infrared Reflectance analyzer (NIR) (Noaman et al., 1984).

Statistical Analyses

Analyses of variance were computed for data from each year and combined over years and soil nitrogen levels. The pooled mean square error was used to test the cultivar mean squares and the cultivar x environment interaction (McIntosh, 1983). All factors were considered fixed. Phenotypic correlations among traits were computed for each year using entry means over soil nitrogen levels. The relationships among traits were analyzed using a multiple regression procedure (Neter and Wasserman, 1974).

The relationships among traits were examined using only cultivars consistent for high and low percent grain protein. A cultivar had high grain protein if it ranked in the top 10 (of 66) in at least four of six soil N environments. A cultivar was considered low grain protein if it ranked in the lowest 10 (of 66) in at least four of six soil N environments. The cultivars were divided by percent grain protein content into three groups:

High grain protein cultivars -- >15% grain protein

Medium grain protein cultivars -- 13-15% grain protein

Low grain protein cultivars -- <13% grain protein

RESULTS AND DISCUSSION

Growing conditions differed between the two years of this study. Environmental conditions were ideal for wheat growth in 1984. The soil nitrogen was high (110 kg/ha) and water stress occurred only late in the grain filling period. In the 1985 experiment the soil nitrogen was low (22 kg/ha) with the drought beginning at heading. Although irrigation was applied at heading in 1985, grain yield was about 50% higher in 1984 (Tables 11-16, Appendix).

Cultivar Variation in Grain Protein and Other Traits

The analyses of variance showed highly significant differences for all traits among cultivars within each year and combined across years. Significant cultivar x environment interactions were attributed largely to years rather than soil N levels. Cultivar mean squares were much larger than cultivar x environment interactions (Tables 7-10, Appendix). In 1984, the check treatment (N0) soil nitrogen content was high (110 kg/ha). Nitrogen added to the soil (N1 and N2) caused no significant changes in grain yield, biological yield, and total plant protein. Grain protein percentage significantly increased with increasing soil nitrogen. In 1985, the soil nitrogen content was low (22 kg/ha) in the check (N0) treatments. Significant differences for all measured traits were obtained among nitrogen levels.

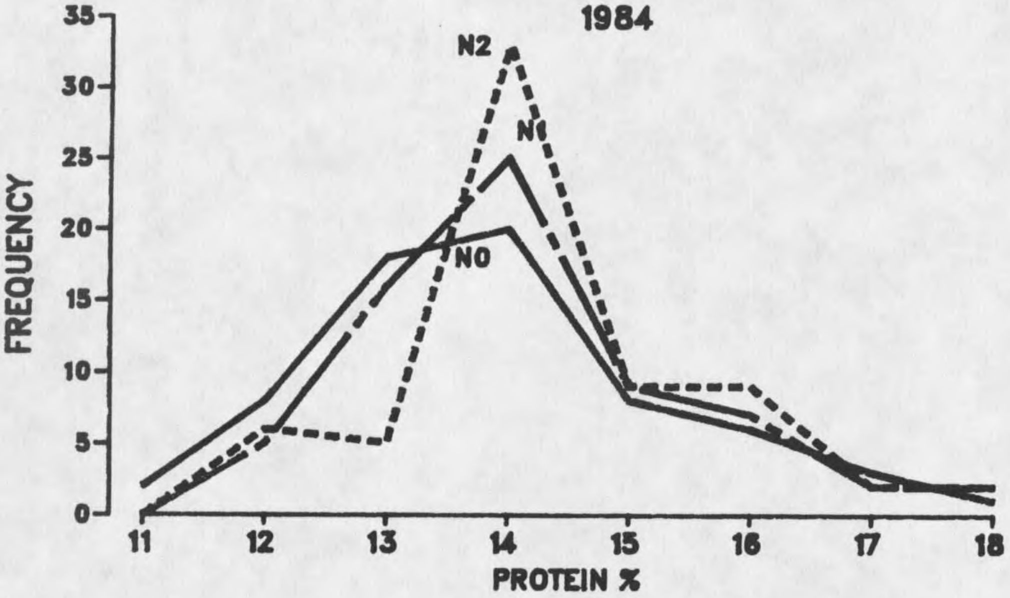

Figure 1. Frequency distribution of percent grain protein for 66 cultivars at 3 soil N levels (1984).

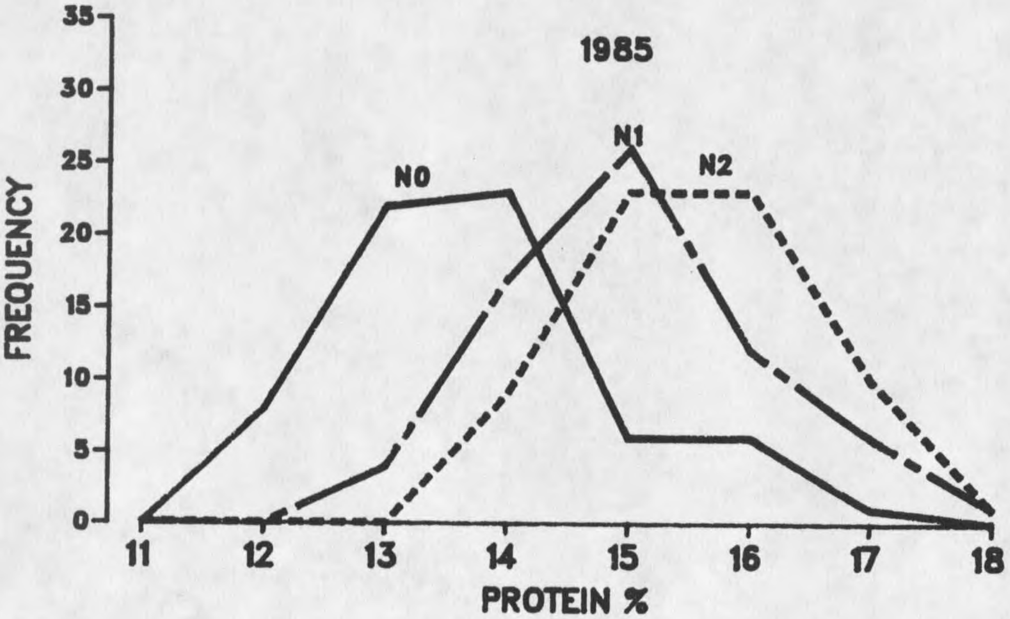

Figure 2. Frequency distribution of percent grain protein for 66 cultivars at 3 soil N levels (1985).

This suggested genetic variability in the elite international winter wheats for grain yield, percent grain protein, and other traits evaluated. Percent grain protein of the 66 cultivars was symetrically distributed around the means in 1984 for all three soil nitrogen regimes (Figure 1). The low protein cultivars increased their protein content with increased soil N, while few changes in grain protein content occurred in the high grain protein cultivars. Although the analysis of variance revealed significant differences among soil N levels, the range of grain protein content was the same at three soil N levels (from 11 to 18%).

Percent grain protein of the 66 cultivars in 1985 was symetrically distributed around individual means for each of the three soil N levels (Figure 2). Increased soil N significantly increased mean percent grain protein. The range and the variation for percent grain protein were similar at the three soil N regimes.

The significant differences for grain protein content in 1984 and 1985 among cultivars and the wide range of grain protein at all soil N levels (Figures 1 and 2) suggested differing genetic capacities for accumulation of protein in the grain. This is supported by the high heritability of percent grain protein in wheat (Stuber et al., 1962; Loffler and Busch, 1982; Cox et al., 1985a). Frequency distributions of grain protein content in the 66 cultivars approaching normality at all soil N levels could be an evidence for quantitative gene control of percent grain protein in these cultivars.

Relationships Among Traits

Dry Matter

Positive correlations were found between biological yield and grain yield in both years (Table 2). Biological yield and percent grain protein were not correlated. Thus selection for high biological yield could improve grain yield without reducing grain protein. Significant increases in grain yield without reduction in percent grain protein were achieved by selection for biological yield (Loffler et al., 1982).

Table 2. Correlation coefficients (r) among trait means of 66 cultivars over 3 soil N levels in two years.

	Biological Yield	Grain Yield	Harvest Index	% Grain Protein	Total Plant Protein
Grain Yield	0.96 ** + 0.97 ** @				
Harvest Index	0.14 ns 0.17 ns	0.42 ** -0.06 ns			
% Grain Protein	-0.04 ns -0.03 ns	-0.27 * -0.09 ns	-0.76 ** -0.20 ns		
Total Plant Protein	0.97 ** 0.97 **	0.91 ** 0.96 **	0.07 ns -0.07 ns	0.13 ns 0.13 ns	
Grain Protein Yield	0.97 ** 0.95 **	0.93 ** 0.97 **	0.15 ns 0.01 ns	0.21 ns 0.16 ns	0.99 ** 0.99 **
N Harvest Index	0.03 ns -0.24 ns	0.19 ns -0.11 ns	0.60 ** 0.52 **	0.22 ns 0.25 *	-0.10 ns -0.17 ns

*, ** = Significant at P<0.05 and <0.01, respectively
ns = Not significant
+ = 1984
@ = 1985

The correlation between grain yield and grain protein percentage was negative and significant in 1984, and not significant in 1985 (Table 2).

Consistent high or low grain protein across environments is important in wheat breeding. Therefore, the relationships among traits were examined using only cultivars consistent for high and low grain protein, deleting the intermediate groups causing an increase in cultivar x environment interactions. The grain yield and percent grain protein of high and low grain protein cultivars are plotted in Figure 3.

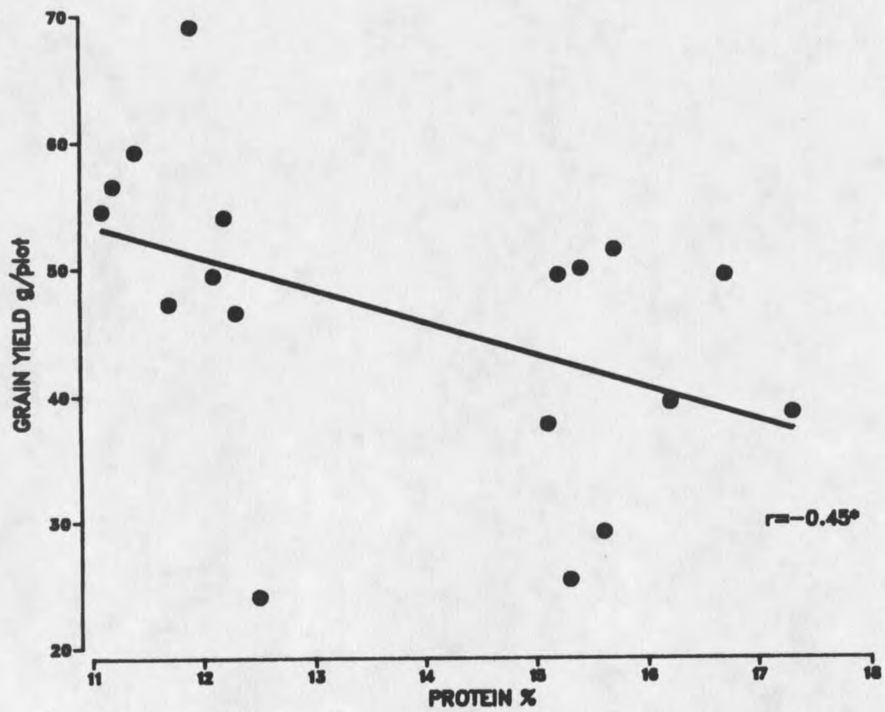

Figure 3. The relationship between grain yield and grain protein (%) for high and low grain protein cultivars over 3 soil N levels (1984).

Despite the negative correlation between grain yield and grain protein, cultivars with high percent grain protein and reasonably high grain yield were identified (Figure 3). The simultaneous improvement of both grain yield and grain protein percentage could be achieved by selection. Similar conclusions were reported by Loffler et al. (1985) and Halloran (1981).

Negative correlations between percent grain protein and harvest index of -0.76** and -0.20 were noted in 1984 and 1985, respectively (Table 2). High grain protein cultivars had low harvest index and vice versa (Figure 4). Selection for high harvest index to improve grain yield could result in reduced grain protein percentage. Similar high negative correlations between harvest index and grain protein were

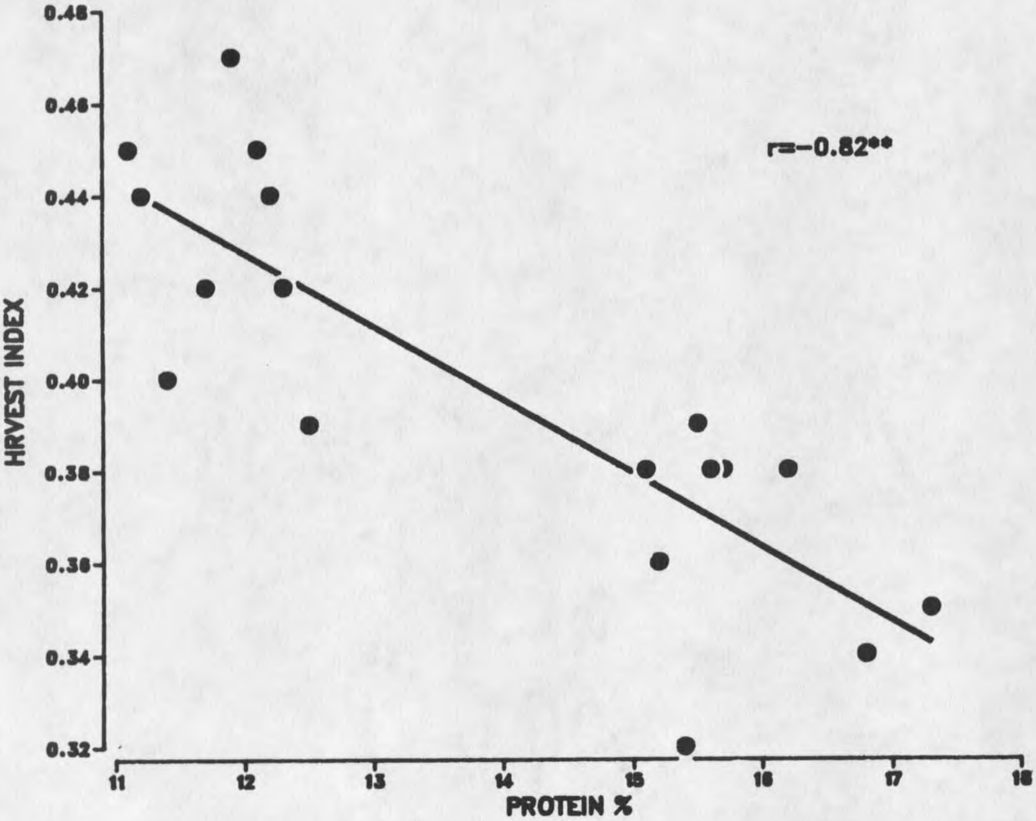

Figure 4. Grain protein (%) and harvest index for high and low grain protein cultivars over 3 soil N levels (1984).

reported by McNeal et al. (1968), Bhatia (1975), and Loffler et al. (1982). They suggested as HI increases, the biomass of vegetative plant parts which serve as an N reservoir decreases. Therefore, less nitrogen is available for translocation to the developing grains.

Plant Protein

Total plant protein accumulated in above-ground parts of the wheat plants was positively correlated with biological yield and grain yield, but not correlated with percent grain protein in 1984 and 1985 (Table 2). Selection for high total plant protein could increase grain yield without decreasing grain protein. Total plant protein was strongly associated with both grain yield and grain protein yield accounting for 83 to 98% of their variation (r = 0.91 and 0.99, respectively; Table 2). This is in agreement with Neales et al. (1963) and Cox et al. (1985b).

Nitrogen harvest index is the genotype's ability to partition nitrogenous compounds between the grain and the vegetative plant parts. The NHI values from 0.60 to 0.83 are within the range reported by Dubois and Fossati (1981) for winter wheat cultivars. This range was higher than that reported by Halloran and Lee (1981), and Loffler et al. (1985), for spring wheat cultivars. Significant positive correlations between NHI and harvest index of 0.60 and 0.53 were found in 1984 and 1985, respectively (Table 2).

This suggests translocation of carbohydrate and nitrogenous compounds are associated. The correlation between NHI and percent grain protein ($r = 0.25$ $P<0.05$) in 1985 was significant but low, and not significant in 1984 (Table 2). Factors other than NHI are important for grain protein percentage. However, the high grain protein cultivars had higher NHI than the low grain protein cultivars (Figure 5). Grain yield

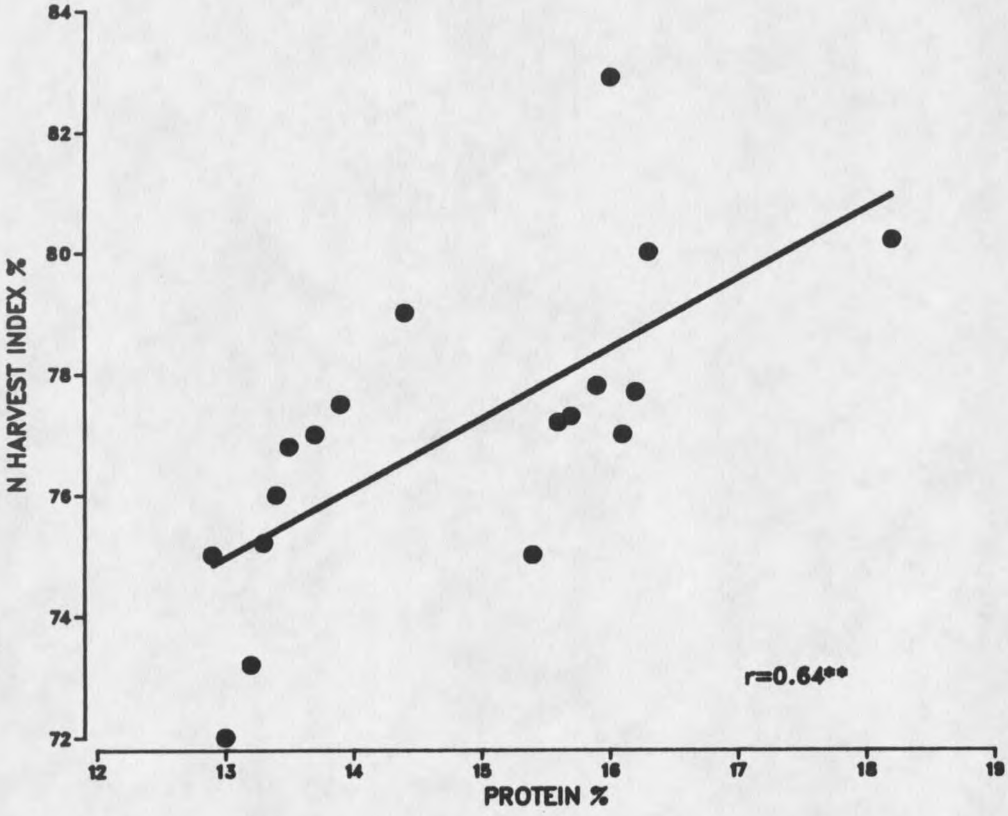

Figure 5. N harvest index and grain protein (%) for high and low grain protein cultivars over 3 soil N levels (1985).

was not correlated with NHI in either year. A greater efficiency for translocation of nitrogen to the grain should result in increased grain protein content at existing grain yield levels. These results are in contrast with those reported in other wheat studies (Cox et al., 1985; Loffler et al., 1982). They found NHI was positively correlated with grain yield but not correlated with grain protein. They concluded selection for high NHI could increase grain yield without reduction in grain protein. However, Halloran and Lee (1979) suggested selection for