THE INHERITANCE OF NITRATE REDUCTASE ACTIVITY AND ITS CORRELATION WITH GRAIN PROTEIN IN A HARD RED WINTER WHEAT CROSS

By

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CHAPTER I

INTRODUCTION

The major problem of nutrition in the world today is related primarily to a shortage of protein. Since cereals have been and will continue to be the mainstay of the world protein supply, more emphasis must be placed on the development and utilization of genetically improved plants of high protein content. This calls for a wider research effort into the genetic and biochemical mechanisms governing protein synthesis in food crops.

The importance of wheat as a world food crop is formidable. Until recently, however, little has been done to increase the protein content in this important cereal grain. Emphasis generally has been placed on increasing yields while holding protein content steady. This does increase the protein production per acre but results in no increase of the protein content of the kernel.

Since it is now accepted that the mechanism of gene action is at the biochemical level through the control of enzyme synthesis, plant breeders cannot accurately assess the possibility of manipulating the protein content in crops such as wheat until a more thorough understanding of the physiologic and biochemical basis of high protein content becomes known.

Studies on nitrate reductase, the first enzyme in the pathway for the reduction of nitrate and subsequent synthesis of protein, might

provide an insight into the complex interaction of genotype-environment-metabolism phase of protein production.

The objectives of this study were (a) to estimate the heritability of nitrate reductase activity in a wheat cross; (b) to estimate the heritabilities of grain protein content and straw protein content in this same cross; (c) to determine the correlations of nitrate reductase with several related plant characters; and (d) to study the effects associated with protein translocation from the vegetative tissues.

CHAPTER II

LITERATURE REVIEW

One of the first reports on breeding wheat for high protein content was by Clark (6) in 1926. He found grain protein negatively correlated with yield and suggested that yield was the most important single factor in determining grain protein. Although the data indicated the inheritance of grain protein was as complex as that of yield, he concluded that the total amount of grain protein per acre could be increased by selecting for improvement in yield while maintaining the protein content of the high-protein parent.

Since then, numerous studies have been conducted showing a generally negative relationship between grain protein and yield. Stuber, et al. (33) working with a cross of 'Atlas 66' and 'Wichita', two varieties differing considerably in protein content, found grain protein negatively correlated with plant height, tiller number, yield per head, and yield per plant. A broad sense heritability value of .678 was estimated by using the P_1, P_2, and F_1 variances. The data did not indicate a preponderance of genes for either high or low protein content but did indicate polygenic control.

In a similar study, Davis, et al. (9) using four populations from soft winter wheat crosses involving the high protein variety Atlas 66 found heritability estimates for grain protein ranging from .54 to .69 indicating the presence of considerable genetic variability.

Correlations between grain protein and yield in 3 of the 4 populations were negative. The ratio of population means to parental means indicated partial dominance for low protein.

Haunold, et al. (17) working with Atlas 66 X Wichita and Atlas 66 X 'Comanche' populations obtained heritability estimates of .36 and .25, respectively, from parent-offspring regression techniques. Broad-sense and narrow-sense estimates of .56 and .28, respectively, were calculated for the Atlas 66 X Comanche population grown under greenhouse conditions.

In a study by Gilmore, et al. (12), results indicated that a predicted genetic gain of 0.66% protein could be expected from selecting the upper five percent of F_4 families grown in single plots with one protein analysis per family. The genetic gain was predicted using mean estimates of variance components for F_2 and F_4 families from ten crosses considered the type a plant breeder would make to increase percent protein. The mean genetic variance of F_2 families in the F_3 was .322, $\pm$ .037, and the mean genetic variance of F_4 families in the F_5 was .309, $\pm$ .029.

Johnson, et al. (24) have stated that there are two primary reasons for the lack of progress in improving the protein content of wheat during the last 60 years. First, the known genetic differences in grain protein among varieties of common wheat were small in comparison to environmental differences. Secondly, superior genetic sources of high protein within T. aestivum were not identified until 1950. They also stated that associated with the trend for higher yields has been the trend toward lower protein in the grain. They reported that particular emphasis is now being placed on the development of hard red winter wheat varieties capable of producing grain of higher protein content

than currently grown varieties either through more efficient extraction of available nitrogen from the soil or by translocating more nitrogen from the plant to the grain.

Effects of Nitrogen on Grain Protein

Grain protein expressed as a percentage value represents the ratio of protein to non-proteinaceous material in the grain. Therefore, a change in either component will affect the magnitude of the percentage value. Since protein in the grain is the result of translocation of nitrogenous compounds from other parts of the wheat plant, the nitrogen level in the plant should presumably influence the amount of nitrogenous materials translocated to the grain.

Haunold, et al. (18) found that at low levels of soil nitrogen availability, grain protein was negatively correlated with yield. The existence and operation of an internal protein-fixing threshold was also found and appeared to be operative in the absence of soil nitrogen limitations. The threshold represents the maximum level of protein attainable in the grain independently of yield of a variety. A threshold for Atlas 66 at least 3% higher than Wichita was indicated.

Terman, et al. (34) found highly significant yield-protein relationships in wheat grain at each level of applied nitrogen in an irrigation-nitrogen rate experiment on hard red winter wheat conducted over a three year period. The chief effect of nitrogen application combined with adequate water was to increase yields while the chief effect with severe water deficits was to increase protein content. In intermediate situations, nitrogen application increased both yield and protein content. Only when nitrogen was absorbed by the plant in excess

of vegetative needs did an increase in protein content of the forage and grain occur. They concluded that differences in inherent protein content among varieties can be shown more clearly under conditions where applied nitrogen results largely in increased yields than where it results largely in increased protein content.

Physiologic Aspects of Plant Breeding

Reitz (29) has stated that there are two ways for yield expression to be viewed: (a) vigor and eventual translocation of metabolites to the kernel; and (b) protection against interference with metabolic processes. The former involves rates of DNA and RNA synthesis, cell division, enzyme activities, photosynthetic rates, and efficiency of translocation. He stated that since wheat ranks relatively low in photosynthetic efficiency among crops, more emphasis is now being placed on physiological efficiency and altered plant morphology.

In discussing the development of "crop ideotypes", Donald (10) proposed the incorporation into breeding programs of "model characters" such as short stems, erect leaves, density tolerance, or any other plant character involving the anatomy or physiology of a crop species.

Hageman, et al. (15) stressed the concept that plant growth and grain yield are the end result of a series of biochemical reactions, each of which is catalyzed by specific enzymes. Determination of the major enzymatic controls involved in the fundamental metabolic systems could pave the way for development of superior genotypes through highly efficient selection techniques.

Studies on nitrate reductase (NR) could provide an insight into the complex interaction of the genotype-environment-metabolism phase of

physiologic production of grain protein.

Role of NR in Grain Protein Production

Recent evidence indicates that NR plays a major role in regulating nitrogen metabolism in cereal crops. It is (a) the first enzyme in the pathway for the reduction of nitrate; (b) substrate inducible (1,2,20); (c) labile *in vivo* under environmental stress (28); (d) variable in level both diurnally and seasonally (13,41); (e) labile in excised seedlings deprived of nitrate or when protein synthesis is inhibited (31); (f) related to total reduced nitrogen accumulated by plants (31); (g) associated with increased protein formation and decreased nitrate content (13); and (h) linearly related to the total grain protein production within a given genotype (8). Also, nitrate accumulates in vegetative plant parts without toxic effects, whereas none of the intermediate compounds between nitrate and amino acids are accumulated according to Hageman, *et al.* (13).

Experiments by Schrader, *et al.* (31) suggest that the enzyme is a single protein moeity capable of dual cofactor utilization. A half-life of approximately 4 hours at room temperature was estimated from inactivation studies. Further evidence indicates NR should be designated NADH:nitrate reductase (E.C. 1.6.6.1).

Numerous studies have been conducted showing the relationship of NR activity to protein production in the plant. In a study with two corn hybrids, Hageman, *et al.* (13) found a diurnal variation in NR activity, water soluble protein, and nitrate content of both hybrids. The diurnal variation of the NR was correlated positively with water soluble protein content and negatively with nitrate content. Also,

plants with the least amount of shading had the highest NR activity which led investigators to believe that reduced yield of corn grain under high populations could be the result of an inadequate level of reduced nitrogen.

Zieserl, et al. (41) found no overall correlation between NR activity and water soluble protein content of four corn hybrids studied, although seasonal protein content paralleled the NR activity with a 7 to 10 day lag period at maximum and minimum points. Even though they could not show a cause and effect relationship between NR, protein, and physiologic stage of development, they found an excellent parallelism between them. Ranking of the four hybrids for NR activity agreed well with their agronomic performance.

Beevers and Hageman (3) found that plants of the Gramineae family treated with 2,4-D showed increased NR activity, increased protein content, and decreased nitrate content. The changes in enzyme activity were positively correlated with changes in protein content.

Hageman and Flesher (14) found that the NR activity in shaded corn plants decreased roughly in proportion to the amount of shading. They found a positive correlation between NR and protein content in both strains studied. They concluded that both light and nitrate are necessary for the formation of NR in quantities required by the plant for normal growth.

Harper and Paulsen (16) found a highly significant correlation (.856) between NR activity and water soluble protein in winter wheat. They found a decrease in NR activity as the plants matured and associated it to a decrease in nitrate uptake; however, they did not rule out tissue ageing and higher temperatures as the casual factor.

Reduced NR activity has been attributed to decreased light, low moisture, low fertility, and genotypic differences. Mattas and Pauli (28) working with young corn plants under moisture stress found that NR activity decreased sharply with short exposures to stress before changes in water content became evident. Decreased activity was reflected in accumulation of nitrate. Moisture stress also increased the incorporation of reduced nitrogen into nonsoluble forms much faster than to soluble protein forms.

Zieserl and Hageman (40) evaluated 47 corn inbreds for NR activity and found up to five-fold differences in enzyme level. Highly significant differences were found in water soluble protein content and in nitrate content. However, no significant correlation was found between NR activity and water soluble protein content, nor was a significant inverse correlation found between NR activity and nitrate content.

Likewise, Toman and Pauli (35), studying NR activity in crowns of winter wheat during cold hardening and dehardening found no significant correlations between nitrate content and NR activity nor NR and water soluble protein content.

It is now well established that NR is a substrate inducible enzyme. Beevers, et al. (2) have further shown that the induction of NR is approximately proportional to the nitrate level in the tissue. Ingle, et al. (22) have provided evidence that induction of NR in radish cotyledons requires the synthesis of RNA and protein. Also, the loss of NR activity depends on de novo protein synthesis as reported by Travis, et al. (36) working with barley leaves. Elsner (11) has shown that induction of NR activity in corn is due to de novo protein synthesis, also. He classified several genotypes differing in (a) initial induction rate

and plateau time; (b) nitrate accumulation level; and (c) *in vitro* decay of NR. Both Travis and Elsner indicated the importance of the balance between the activation and inactivation systems in controlling the enzyme level in plant tissue.

Genetic Control of NR

Zieserl and Hageman (40) evaluated 47 corn inbreds for NR activity and found up to five-fold differences in enzyme level. Seasonal patterns of NR in certain related lines were found to differ markedly, apparently reflecting differences in genetic backgrounds. Although the NR levels were much higher the second year of the study, the seasonal NR patterns were highly comparable for the two years.

In a subsequent experiment by Zieserl, *et al.* (41), the NR levels of 4 corn hybrids were compared to their parental inbreds. Comparison of the seasonal mean NR values indicated a generally additive mode of inheritance.

In a study by Schrader, *et al.* (30), a number of inbred lines of corn were ranked from "high" to "low" with respect to their seasonal mean NR levels. The following crosses were made: "high X high", "high X low", and "low X low". None of the "high X high" F_1 hybrids exhibited higher enzyme activity than the midparent value. The enzyme levels in the "high X low" hybrids were essentially intermediate to the parental inbreds. Two of the "low X low" hybrids exhibited heterosis while the others were not significantly different from the midparent level.

Warner, *et al.* (38) has shown that the level of NR activity in a genetic population of corn was under control of a two-locus system with dominance. Each inbred parent was homozygous for a dominant or

partially dominant allele at one locus and homozygous recessive at a second locus. The high parent, 'B14', carried a dominant allele at one locus while 'OH43', the low parent carried a recessive allele at this locus. The F_1 hybrid involving these two parents possessed a heterotic level of enzyme activity. The data suggested that since the two inbreds differed in their rate of enzyme synthesis and *in vitro* decay, this heterotic level was the result of inheritance of qualities that give the hybrid intermediate rates of synthesis and decay. The data support evidence that genotypic differences in reductase stability and enzyme synthesis rate exist. It is interesting to note that the inbred with the lowest activity, OH43, exhibited the highest rate of both synthesis and decay.

Since NR is a substrate inducible enzyme, it is possible that a regulator gene may be involved in the mechanism or function of these loci. Findings by other workers support this concept.

Croy (7), in comparing two varieties of winter wheat differing in grain protein content, found no close numerical relationship between the average level of enzymatic activity and the average water soluble leaf protein content, which was as expected. However, the data did show a significant positive correlation between the input of reduced nitrogen estimated from the enzyme assays and the accumulation of grain protein for both varieties. The enzymatic activity was positively correlated with water soluble protein within and between the genotypes and with grain protein within the genotypes. The high protein variety, 'Ponca', had a higher level of NR activity than the low protein variety, 'Monon', both years of the study. These differences were small but considered to be real.

In another part of the study, 32 varieties of winter wheat were analyzed for NR at 6 separate sampling dates. Little variation was found among the genotypes. Only on one date did the varieties significantly differ in their NR levels; however this was the only date when nitrate was present in the tissue in adequate amounts to permit full expression of their genetic potentials.

Croy (7) suggested that lack of genetic diversity was not the cause of minimal progress in developing high protein wheat. Instead, he characterized the inability to isolate and identify the interacting metabolic factors involved in protein production as the main reason for lack of progress.

Translocation Effects

Johnson, et al. (24) implied that if differential uptake of nitrogen was the major factor underlying high protein content of wheat, high availability of soil nitrogen would be required for phenotypic expression of the protein genes. If differential translocation was the basis for high protein grain, then the trait would be expected to express itself phenotypically in an array of production situations. They concluded from the data that more efficient and complete translocation of nitrogen from the plant to the grain was the physiological basis of high protein grain. Plants of 'Warrior', a low protein variety, contained significantly more nitrogen than the high protein 'Selection 60305' and other varieties. The grain of Warrior contained only 56% of the total per plant nitrogen while Selection 60305 contained over 60% of the total per plant nitrogen in the grain.

Hay, et al. (19) in studies with corn, found that (a) the percent

total nitrogen decreased in all vegetative parts of the corn plant from pollination to maturity; (b) the vegetative parts of an average plant contributed approximately 60% of the total grain nitrogen at maturity, the other 40% coming from the soil and roots; (c) evidence favors the view that nitrates were converted into protein or protein-like substances in the plant, then translocated to the grain; and (d) nitrogen in the grain made up approximately two-thirds of the nitrogen of the whole plant including grain.

After reports by Hoener and DeTurk (21) that high protein corn selections accumulated more protein in the vegetative parts of the plant than did the low protein selections, Seth, et al. (32) began a study to determine (a) whether high protein wheat varieties have a higher protein content in the vegetative parts than low protein varieties, and (b) whether high protein varieties utilize a larger proportion of the nitrogenous materials of the vegetative parts to form protein in the kernels.

No differences were found in the nitrogen content of the vegetative parts of high and low protein varieties. Differences in the protein content of the two varieties studied appeared to be associated with a difference in rate of protein synthesis in the developing kernels. However, the data indicated a more rapid transfer of nitrogenous materials from the vegetative parts, especially the roots, to the heads of the high protein varieties.

These results suggested that there was a major difference between corn and wheat in the physiological basis for varietal differences in protein content.

CHAPTER III

METHODS AND MATERIALS

The Genetic Population

The two parent selections used to construct the genetic population were Atlas 66/Comanche ('NB65679') and 'D145B4' (OK6011). Both selections are hard red winter wheats. The two parents were chosen for their apparent difference in grain protein content. D145B4, a "Triumph type", is an early maturing, well adapted selection that generally exhibits rather low protein content. NB5679 is somewhat less adapted to Oklahoma, matures later than D145B4, and exhibits a considerably higher protein content. The NB65679 line was obtained from Dr. V. A. Johnson, U.S.D.A. and Agricultural Experiment Station, Lincoln, Nebraska. D145B4 traces to one of "several" hundred breeder's samples bequeathed to the Oklahoma State University by the late Joseph E. Danne. The two parental lines were not screened previous to this experiment for differences in nitrate reductase activity.

A population consisting of P_1 (NB65679), P_2 (OK6011), F_1 and F_2 plants was constructed by making the necessary crosses in the greenhouse. The experimental population consisted of 32 plants each of P_1, P_2, and F_1 genotypes and 128 F_2 plants. Numbers of plants of each genotype were rather limited because of limitations imposed by laboratory analysis.

The Field Layout

The field layout was a split plot design with eight replications. There were 4 main plots which allowed sampling on 4 different dates to facilitate laboratory analysis. Each of the four main plots contained 7 plants, i.e., 1 P_1, 1 P_2, 1 F_1, and 4 F_2 plants. The subplots consisted of the genotypes. Each subplot contained only one plant each. The statistical design differed from the field design because the parents and F_1 plants were analyzed separately from the F_2 plants.

Cultural Practices

The seeds were planted in the greenhouse in plant bands on September 19, 1969 and seedlings were transplanted to the field on October 7. The plants were space planted 28.5 cm apart bidirectionally.

The field which was located on the Agronomy Research Station Stillwater, had received a preplant application of 44.8-89.6-44.8 kg/ha of N, P_2O_5, and K_2O, respectively. A supplemental application of 11.2-22.4-11.2 kg/ha of N-P_2O_5-K_2O was banded between rows with a small hand planter on October 23. On January 29 and again on February 28, 1970, applications of 56 kg/ha of actual nitrogen (as NH_4NO_3) were applied. On April 8, to bolster enzyme activities, an application of actual nitrogen at the rate of 112 kg/ha was made.

The plants received no irrigation other than that applied immediately upon transplanting.

Sampling Procedures

Plant samples were taken in the fall and spring and assayed for nitrate reductase activity, water soluble protein content, and nitrate

content. Each main plot was sampled on separate days in both spring and fall. The sampling dates in the fall were November 17, 18, 19, and 21. The spring samplings were made on April 3, 16, 24, and May 1. The actual sampling occurred between 8:00 and 9:00 a.m. Only the leaves were collected. All samples were immediately placed in a plastic bag and kept under ice.

Laboratory Procedure

In the laboratory the samples were kept in ice until processed to prevent enzyme denaturation. The samples were ground and extracted according to the procedure outlined by Hageman and Flesher (14) with the following modification. For each gram of ground leaf tissue, 9 ml of extraction solution (0.025 M potassium phosphate, 0.005 M EDTA, and 0.01 M cysteine adjusted to pH 9.0 with KOH) were added.

The NR assay was performed as described by Hageman and Flesher (14) with the following modifications. The assay was conducted at 29 C for 15 minutes and a lower level (0.2 mg) of NADH was used. Water soluble protein content was estimated according to the procedure of Lowry, et al. (26). Nitrate content in the plant extracts was estimated by the procedure described by Wooley, et al. (39).

Grain protein content was estimated according to the standard Udy procedure outlined by Udy (37). Estimation of the percent protein in the straw was accomplished using the micro-kjeldahl procedure.

Statistical Analysis

Statistical analysis was performed for two major aspects of this study: (a) estimation of heritabilities of several important characters,

and (b) determination of phenotypic correlations among certain combinations of characters evaluated in the study.

The data from the F_2 plants were analyzed as a randomized complete block with subsamples. The data from the parents and F_1 plants were analyzed as a split-plot design. The analysis was handled in this manner to obtain valid estimates of genetic and environmental variances.

Estimates of heritability were calculated according to the formula used by Burton (5):

$$H = \frac{VF_2 - \frac{VP_1 + VP_2 + VF_1}{3}}{VF_2}$$

where:

H = broad sense heritability

VF_2 = phenotypic variance of the F_2, which estimates total variance

$\frac{(VP_1 + VP_2 + VF_1)}{3}$ = mean phenotypic variance of non-segregating populations, which estimates environmental variance.

Phenotypic correlation coefficients were calculated from F_2 data only. The pooled phenotypic correlation for any two variables was found by using the sums of squares and cross products for the F_2 in reps in dates obtained in the analysis of variance. Results of the fall sampling period were not used in computing the correlations.

CHAPTER IV

RESULTS AND DISCUSSION

Genetic Variability for NR Activity and Grain Protein Content

Genetic variability must exist within a base population if a plant breeder is to make progress based on selection of phenotypes. A more detailed discussion on the implications of selection and heritability will be presented in a later section, but first, existence of genetic variability for the characters studied must be demonstrated. This can be accomplished by presenting the character means for the parents, the F_1, and the F_2.

The results of the NR assays in the fall of 1969 and spring of 1970 are presented together with grain protein content in Table I. These data are also shown graphically in Figures 1 and 2. From Table I, the following points are of significant interest: (a) on all four sampling dates in the spring sampling period the high protein parent, NB65679, possessed higher NR levels than did the low protein parent, D145B4; (b) this difference in activity between the two parents was significant at the .01 level of probability for the spring data; (c) the F_1 and F_2 generation responses were intermediate to those of the parents of all four dates for NR activity in the spring; (d) on only one sampling date in the fall sampling period did the two parents significantly differ (.05) in their enzyme levels; (e) NB65679 (P_1) demonstrated approximately three percent higher grain protein content than did D145B4 (P_2).

TABLE I

CHARACTER MEANS FOR NR ACTIVITY (FALL AND SPRING) AND GRAIN PROTEIN CONTENT OF THE PARENTS AND PROGENY ON INDIVIDUAL SAMPLING DATES

Sampling Date	NR Activity (fall) μ moles KNO_2/g.f.w./hr.				NR Activity (spring) μ moles KNO_2/g.f.w./hr.				Grain Protein Percent			
	P_1	P_2	F_1	F_2	P_1	P_2	F_1	F_2	P_1	P_2	F_1	F_2
1[1]	4.25	4.84	3.98	4.03	1.78	1.28	1.33	1.54	16.1	13.0**	14.1	14.4
2	3.26	3.02	3.38	2.76	1.36	0.71*	0.75	0.99	16.6	12.9**	14.6	15.1
3	5.19	5.69	5.83	5.20	2.13	1.58*	1.79	2.12	16.1	13.2**	14.4	14.5
4	14.10	11.05*	14.65	12.96	2.18	2.04	2.11	2.39	16.0	13.0**	14.2	14.3
Mean[2]	6.70	6.15	6.96	6.07	1.86	1.40	1.50	1.76	16.2	13.1	14.3	14.6

*P_1 - P_2 contrast is significant at the 0.05 level of probability.

**P_1 - P_2 contrast is significant at the 0.01 level of probability.

[1]Average of 8 responses for P_1, P_2, and F_1, and 32 responses for the F_2 for each sampling date.

[2]Average of 32 responses for P_1, P_2, and F_1, and 128 responses for the F_2.

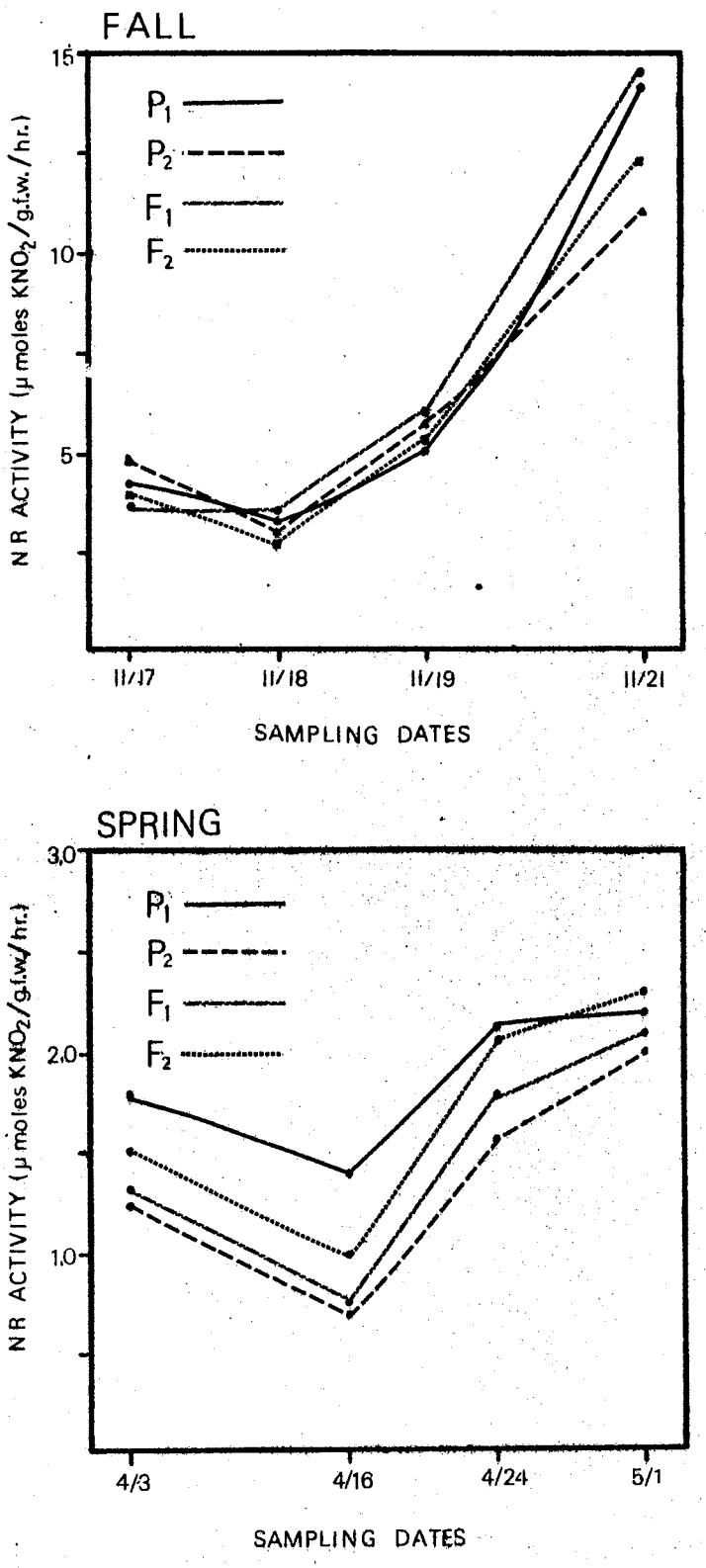

Figure 1. Patterns of NR Activity for NB65679 (P_1), D145B4 (P_2), F_1, and the F_2 During Fall 1969 and Spring 1970 Sampling Periods

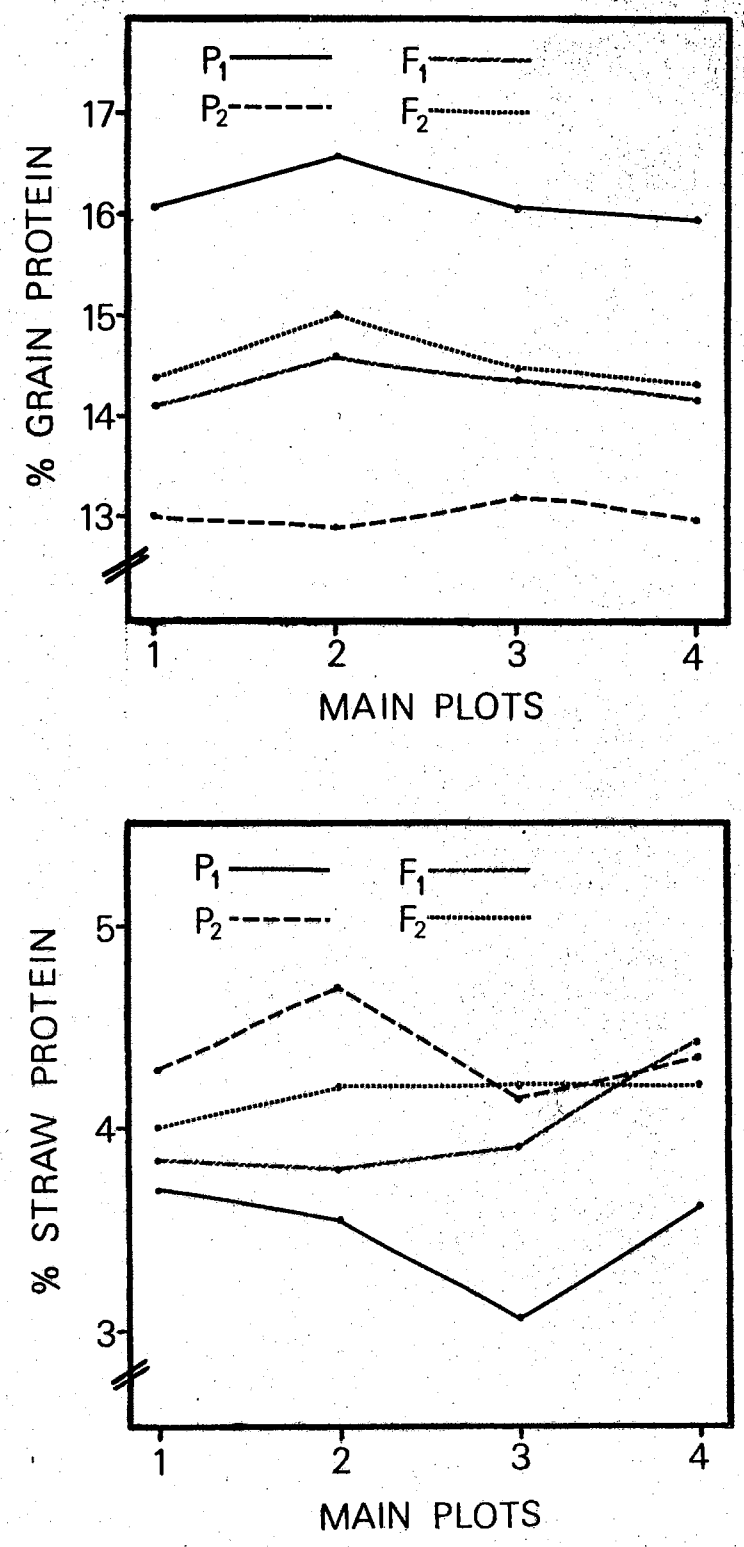

Figure 2. Distributions for Grain Protein Content and Straw Protein Content in the Genetic Population; enzyme sampling dates for the main plots were April 3, 16, 24, and May 1, 1970.

This difference was significant at the .01 level for each main plot; and (f) the F_1 and F_2 generation means for grain protein content were approximately intermediate to the means of the parents with perhaps an indication of slight dominance for the low protein character.

Differences among sampling dates in the fall for NR activity were due more to environmental conditions than to physiological differences since the entire sampling period spanned only five days. It is assumed that significant difference in NR activity that occurred between the parental lines on the fourth date was due to maximum genetic expression of the genes for high NR activity on that date. This date was characterized by conditions which led to maximum induction of NR activity as is evidenced by the nearly three-fold increase in activity as compared to the three previous dates.

The NR activity levels for the spring sampling period were somewhat lower than those of the fall. Spring sampling was initiated later than anticipated, and the NR levels had apparently already decreased due to tissue ageing and higher temperatures. The spring sampling period began on April 3 and ended on May 1. On April 8, after the first sampling date, nitrogen (as NH_4NO_3) was applied at the rate of 112 kg/ha of actual nitrogen. Apparently this was the reason the NR levels did not steadily decrease as the plants approached maturity. The physiologic stages for the four sampling dates were roughly four weeks prior to heading, two weeks prior to heading, less than one week prior to heading, and during heading. Heading (75% of heads exerted beyond flag leaf) occurred on April 29 for D145B4 (P_2) and on May 5 for NB65679 (P_1).

Results from the grain protein analysis indicated no preponderance

of dominant genes for low protein content as shown by population means. This agrees with findings of most other workers. Differences in protein content among the main plots (sampling dates for NR) can be attributed to experimental error plus some effects due to removal of leaf tissue at different physiologic stages of the plants. Drought stress experienced during the later stages of grain filling may have influenced the grain protein content somewhat.

Due to the large dependence of NR activity upon environmental conditions (i.e., light, temperature, nitrate, moisture), it might be expected that on certain days the genetic potential would not be as fully expressed as on other days. This line of reasoning could also apply to the concept set forth by Warner, et al. (38) showing that NR activity in maize was due to differential rates of both synthesis and decay of the enzyme. Therefore, it was assumed that measurable genetic differences for NR activity existed during the early growth stages, and that these differences were more pronounced as the plants apporached maturity.

Relationships of NR to Grain Protein and Other Plant Characters

The phenotypic correlation coefficients between NR activity and grain protein content, water soluble leaf protein, nitrate content, yield, and straw protein are presented in Table II. These correlation coefficients are based on F_2 responses and are a measure of the phenotypic correlations between each of the two characters on each sampling date. Correlations were not computed for the fall sampling date since genetic differences were not as apparent as in spring. The correlation coefficients for each sampling date were tested for homogeneity. Except for correlations involving nitrate, the correlations were found to be

TABLE II

PHENOTYPIC CORRELATIONS INVOLVING NITRATE REDUCTASE ACTIVITY AS DETERMINED FROM F_2 PLANTS

Correlation Between NR and:	Sampling Date[1]				Pooled[2]
	1	2	3	4	
Grain Protein	0.290	0.222	0.580**	0.444*	0.402**
Water Soluble Protein	0.580**	0.139	0.304	0.464*	0.353**
Nitrate Content	-- [3]	0.357	0.530**	-0.145	-- [4]
Yield	0.149	0.134	0.129	0.006	0.096
Straw Protein	-0.026	-0.177	0.063	0.015	-0.015

*Significant at the 0.05 level of probability.

**Significant at the 0.01 level of probability.

[1]Significant values are 0.388 and 0.496 for the 0.05 and 0.01 probability levels, respectively, for 24 degrees of freedom.

[2]Significant values are 0.200 and 0.262 for the 0.05 and 0.01 probability levels, respectively, for 96 degrees of freedom.

[3]Nitrate content could not be measured on Date 1.

[4]Pooled correlation was not computed due to lack of homogeneity.

homogeneous enough to allow pooling.

Most workers have found it difficult to demonstrate significant correlations between NR activity and water soluble leaf protein content due to the lag period that exists and other complexities of the system as indicated by Zieserl, et al. (41). However, on all four spring sampling dates, NR activity was positively correlated with water soluble protein, with a 0.01 probability level on date 1 and a 0.05 probability level on date 4. Also, the pooled correlation coefficient was significant at the 0.01 probability level.

NR activity was also positively correlated with grain protein content on all four sampling dates. These correlations were significant (.05) on the third and fourth sampling dates, and the pooled phenotypic correlation was significant at the 0.01 probability level. These results indicate that NR levels are positively associated with both water soluble leaf protein and grain protein content. These are the expected results assuming no differential influence by internal regulating factors in the system.

The nitrate content in the vegetative tissue was so low that it could not be measured on the first sampling date, but following an additional application of nitrogen on April 8, nitrate levels were measureable on the three remaining dates. On the second and third sampling dates NR activity was positively correlated with nitrate content, but on the fourth date a negative correlation was found. This can best be explained by referring to Figure 3. The nitrogen application applied on April 8 apparently increased the nitrate content in the vegetative tissues of the F_2 plants between the second and third sampling date but leveled off after date 3. Since NR is substrate inducible

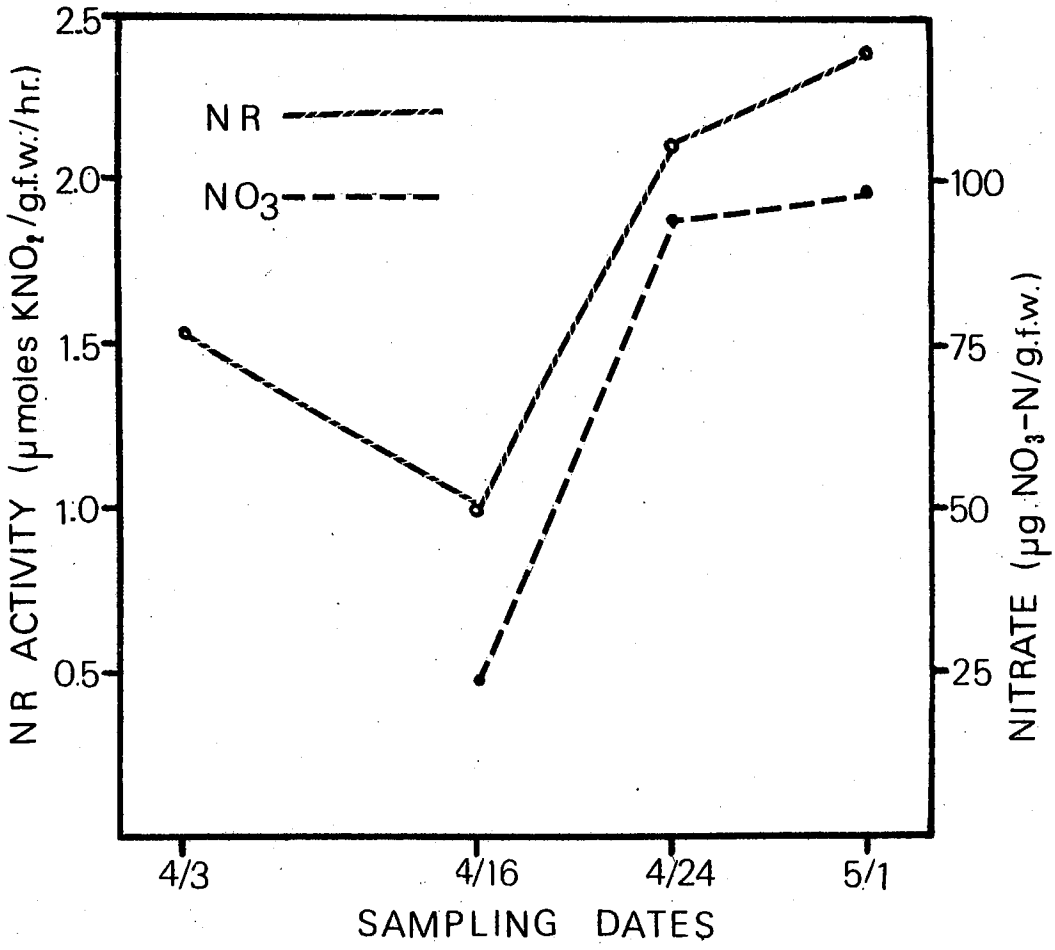

Figure 3. Relationship of Nitrate Reductase Activity to Nitrate Content in the Leaf Tissue of F_2 Plants During Spring Sampling Period. (Nitrate content was too low to measure on Date 1.)

quantitatively, increases in NR activity should be positively correlated with nitrate uptake since induction was occurring (dates 2 and 3). However, after the nitrate uptake leveled off (date 4) one would expect a negative relationship since a plant with higher NR activity should possess less nitrate as found by Zieserl, et al. (41) and other workers. NR activity was positively correlated with yield on each of the four sampling dates, but these correlations were not statistically significant. These positive correlations agree with the findings of Hageman and Flesher (14) in relating NR activity to yield under imposed conditions of reduced light.

Since NR activity maintained a positive relationship with both water soluble leaf protein and grain protein content in this study, high NR levels might be expected to correlate phenotypically with high straw protein content. The negative but statistically non-significant correlations indicate that the amount of protein left in the straw at maturity is influenced more by differential translocation of the nitrogenous material than by increased nitrogen reduction. This concept is consistent with data of Johnson, et al. (24) indicating that high protein lines are more efficient at translocation than low protein lines.

Relationships of Grain Protein to Other Plant Characters

Phenotypic correlation coefficients involving grain protein content are presented in Table III. These correlations are based on the performances of individual F_2 plants. The correlation coefficients varied considerably in magnitude among the main plots. This effect was due in part to differences in sampling with regard to NR activity as described above. The following results were obtained for grain protein

TABLE III

PHENOTYPIC CORRELATIONS INVOLVING GRAIN PROTEIN CONTENT AS DETERMINED FROM F_2 PLANTS

Correlation Between Grain Protein and:	Sampling Date[1]				Pooled[2]
	1	2	3	4	
NR Activity	0.290	0.222	0.580**	0.444*	0.402**
Specific Activity	0.331	0.229	0.404*	0.437*	0.353**
Water Soluble Protein	0.078	-0.133	0.494*	0.103	0.173
Straw Protein	-0.313	-0.047	0.279	0.004	-0.030
Yield	-0.080	-0.569**	-0.273	-0.140	-0.267**
Heading Date	0.403*	0.270	0.280	0.543**	0.356**

*Significant at the 0.05 level of probability.

**Significant at the 0.01 level of probability.

[1]Significant values are 0.388 and 0.496 for the 0.05 and 0.01 levels of probability, respectively, for 24 degrees of freedom.

[2]Significant values are 0.200 and 0.262 for the 0.05 and 0.01 levels of probability, respectively, for 96 degrees of freedom.

correlations: (a) grain protein content showed a highly significant positive correlation coefficient with NR activity, heading date, and specific activity for the pooled data, (b) grain protein was negatively correlated with yield with a 0.01 level of probability; and (c) based on the pooled data no statistically significant correlation was found between grain protein and straw protein content, nor for water soluble protein. However, water soluble protein had a significantly positive correlation with grain protein on date 3.

The correlations between grain protein and NR activity were discussed in the previous section. Approximately the same results were found for the correlations between grain protein and the specific activity of the enzyme. These correlations were positive on all four sampling dates, and significant at the 0.05 probability level on dates 3 and 4. These correlations from the pooled data were significant at .01 level of probability.

Water soluble leaf protein was significantly correlated with grain protein on the third sampling date, the date when the highest correlation between NR and grain protein occurred. However, it could not be determined from this study whether this effect was due to higher NR levels or to mobilization of water soluble leaf protein for translocation. The pooled correlation coefficient for the association between water soluble leaf protein and grain protein was positive but non-significant.

The negative correlation between grain protein and yield agrees with the finding of several workers (6,9,33). The pooled correlation coefficient for this comparison was significant at the 0.01 probability level. It is interesting to note, however, that in this study the yield

of the high protein parent exceeded that of the low protein parent. The correlations, though, were based on the F_2 plants and in this population yield was negatively correlated with protein. Although negative correlations generally indicate that selection for increase in one character could result in decrease in the second character, selection for yield and high protein should not be ruled out based on the performance of the parents in this study.

Grain protein was also positively correlated (0.01 probability level) with heading date indicating that late maturity is associated with the high protein character in this population.

Johnson, et al. (23) has reported that the physiologic basis for high grain protein lies in the more complete and efficient translocation of nitrogenous materials out of the vegetative tissues and into the grain. If this were the case in the present study, negative correlations between grain protein and straw protein would be expected. Based on the straw protein content of the two parents, the degree of nitrogen translocation from the vegetative parts of the plant to the grain does appear to be quite prominent. However, the phenotypic correlation based on the F_2 population did not substantiate this concept. A small overall negative correlation coefficient was obtained, but this was not statistically significant. One possible explanation for this is that removal of leaf samples for enzyme assays may have altered normal translocation patterns. This also raises the question of which is more important in grain protein production, high nitrogen reduction or efficient protein translocation.

Grain Protein Interrelationships

The mean values for grain protein content, straw protein, and NR activity (spring analysis) are presented in Table IV. It is of interest to note that the high protein parent (P_1) exhibited the highest level of NR activity and the lowest level of straw protein content, both these levels being significantly different from the low protein parent. The low protein parent (P_2) possessed the lowest level of NR activity and the highest level of straw protein content. The F_1 and F_2 populations were intermediate to the two parents for both characters. These data support the concept that increased grain protein content is due to both increased nitrogen reduction throughout the growing season (or at least during the critical period) and more complete and efficient translocation of protein out of the vegetative tissues and into the developing kernels.

Perhaps if this concept were researched in a more quantitative manner (i.e., by estimating the total seasonal reduction of nitrate and measuring the quantitative differences resulting from translocation of protein) more pertinent information could be ascertained as to the significance of each of these systems in determining grain protein production.

Heritability Estimates

Heritability in the broad sense is generally defined as the ratio of the total amount of genetic variation to the total phenotypic variation. A knowledge of the inheritance of any character is desired to enable the modern plant breeder to effectively and efficiently manipulate the genotype by selection from a variable source population.

TABLE IV

PARENTAL, F_1, AND F_2 CHARACTER MEANS FOR NR ACTIVITY, GRAIN PROTEIN, AND STRAW PROTEIN

Genotype	NR Activity[1] μ moles KNO_2/g.f.2./hr.	Grain Protein Percent	Straw Protein Percent
P_1[2]	1.86	16.18	3.49
P_2	1.40**	13.05**	4.38**
F_1	1.50	14.34	3.99
F_2[3]	1.76	14.57	4.16

**P_1 - P_2 contrast is significant at the .01 level of probability.

[1]Includes results from spring sampling only.

[2]Mean values for P_1, P_2, and F_1 are averages of responses from thirty-two plants each.

[3]Mean values for F_2 are averages of responses from 128 plants.

Several workers have discussed the concept of heritability and the implications involved in efficient selection programs (4,5,25,27). The heritabilities estimated in this study were based on the method reported by Burton (5) using the variance from the genetically homogeneous populations to estimate the environmental variance and the variance from the segregating (F_2) population to estimate total variance.

Heritability estimates for grain protein content, NR activity, straw protein, yield, and heading date are presented in Table V. Heritabilities were estimated for the individual sampling dates (main plots) since the genetic expression of the NR enzyme apparently is quite sensitive to different environmental and physiological conditions. Differences in the heritability estimates of the characters other than NR are also apparent. It is thought that these differences arise partly because of removal of sizeable amounts of leaf tissue during sampling at different physiologic stages which might interfere with genetic expression of grain protein, straw protein, and yield.

The pooled estimate of heritability of 44.0% for grain protein compares with the heritability estimates reported by Haunold, et al. (17) for the Atlas 66 X Comanche population. The higher estimate of heritability of 75.5% for grain protein on the first sampling date approaches the high estimate reported by Stuber, et al. (33) working with Atlas 66 X Wichita. The differences between the high estimate for the first sampling date compared to the rather uniform and somewhat lower estimate on the last three dates might be due in part ot the application of 112 kg/ha of actual nitrogen after the main plots for the first sampling date had been clipped and the other three had not. The effects of large amounts of supplemental nitrogen on expression of genetic

TABLE V

ESTIMATES OF HERITABILITY

Character	Sampling Date[1]				Pooled[2]
	1	2	3	4	
Grain Protein	75.5	32.1	30.3	34.6	44.0
NR Activity	-03.2[3]	-39.6[3]	71.7	24.8	29.8
Straw Protein	48.1	57.4	20.2	-38.7[3]	27.7
Yield	13.6	25.3	18.0	46.5	25.8
Heading Date	70.3	71.7	85.6	50.9	72.3

[1]Sampling dates for NR assays were April 3, April 16, April 24, and May 1, 1970. Estimates for heritability for each sampling date were based on 24 responses for homogeneous genotypes and 32 responses for heterogeneous genotypes for each character.

[2]Estimates of heritability were based on 96 responses for homogeneous genotypes and 128 responses for heterogeneous genotypes for each character.

[3]Indicates estimate of zero.

differences for protein production as reported by Haunold, et al. (18) may have biased these heritability estimates downward. Perhaps a genetic study specifically designed for grain protein alone would give more accurate and reliable estimates of heritability for this trait in this population. One of the purposes of this study, however, was to assess the possibility of basing selection on more than one character for maximum success in developing high protein lines.

It should be pointed out that the negative estimates occurring in Table V indicate estimates of zero. These negative estimates apparently occurred because more variance was exhibited by the homogeneous genotypes (P_1, P_2, and F_1) than the heterogeneous F_2 for these particular characters and conditions. This is one of the problems encountered with this particular method for estimating heritability.

In viewing the heritability estimates of NR activity, caution must be exercised in the interpretation of such data. It is fairly obvious that the physiologic stage of development of the plant and environemntal conditions greatly influenced the heritability estimates from one sampling date to the next. It is purely a matter of conjecture whether or not one wishes to view the rather high estimate obtained on date 3 as representing the potential heritability of NR activity. This date was characterized by high levels of NR activity, optimum conditions for maximum induction, and significantly positive phenotypic correlations between NR and water soluble protein and between NR and grain protein. Therefore, it would seem likely that this date does represent the best conditions for expression of maximum genetic potential. The concept presented by Warner, et al. (38) relating NR activity in maize to different rates of enzyme synthesis and enzyme decay, both under genetic

control, may help explain why NR activity expresses such complex genetic control. The pooled heritability estimate of 29.8% and the high single date estimate of 71.7% does show promise that genetic manipulations of this enzyme character could be manifested if more knowledge on optimum conditions for sampling could become known.

The heritability estimates for straw protein content vary somewhat with sampling date as does NR activity. The negative estimates that occurred on the fourth sampling date might be explained by the fact that considerable amounts of leaf tissue were clipped from the plants at a time when maximum translocation was occurring (just after head emergence). This condition could conceivably alter the normal translocation system in the plants and interefere with the normal genetic expression of genes responsible for protein translocation. The two heritability estimates of 48.1% and 57.4% on dates 1 and 2, respectively, would indicate improvement for efficient translocation could be achieved provided the optimum conditions for selection were determined. These straw protein results indicate the feasibility for determining the genetic control of the protease enzymes responsible for translocation.

The rather low heritability estimates for yield compare favorably with those encountered by most workers working with this quantitative trait. However, it should be emphasized that yield differences between the parents were small and yield on a single plant basis was quite variable.

Heading date showed high heritability estimates with a pooled estimate of 72.3%. Considerable dominance for early heading was demonstrated in this population.

In general, estimates of heritability apply to the particular population, and set of environmental conditions from which the estimates were obtained. The heritability estimates reported here apply specifically to this genetic population and the environmental conditions encountered in this study. However, the scope of this study was mainly to provide an insight into the mechanisms involved in high protein wheat and to probe the genetic implications of such mechanisms. Before such bio-chemical criteria could be employed successfully in a plant breeding program, further research should be done to verify these preliminary findings and to pinpoint the optimum conditions for successful selection.

SUMMARY AND CONCLUSIONS

The objectives of this study were: (a) to estimate the heritability of nitrate reductase activity in the NB65679 X D145B4 wheat cross; (b) to estimate the heritability of grain protein and straw protein in the same cross; (c) to determine the correlations among nitrate reductase activity, grain protein, and several related plant characters; and (d) to study the effects associated with protein translocation out of the vegetative tissues.

The parents used to construct this genetic population were found to differ significantly in NR activity, grain protein and straw protein content. Under optimum conditions for NR activity, the heritability estimate for NR activity was found to be 71.7%. Genetic variability was also demonstrated in the fall growth stages for NR. Heritability estimates for grain protein ranged from 30.3% to 75.5% with a pooled estimate of 44.0%. Although quite variable, heritability estimates for straw protein indicated the presence of considerable genetic variance for translocation effects.

NR activity was positively correlated with water soluble protein and grain protein. NR activity was correlated positively with nitrate during induction and negatively with nitrate during normal uptake. Grain protein was negatively correlated with yield and showed a negative relationship with straw protein content.

The high protein content appeared to be due to both higher nitrate reduction and to more complete and efficient translocation of

proteinaceous materials out of the vegetative tissues and into the developing kernels.

The scope of this study was preliminary in nature, to provide insight into the possible uses of such bio-chemical criteria in a plant breeding program. These findings indicate the strong possibility that information on nitrate reductase activity can be successfully used by plant breeders to increase the protein content of wheat.

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