

NITROGEN ASSIMILATION AND PROTEIN SYNTHESIS IN LOW- AND
HIGH-PROTEIN LINES OF WHEAT AND OTHER GRAIN SPECIES

by

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INTRODUCTION

Plant proteins constitute the primary source of amino acids in the human diet, not because of nutritive advantage, but because of a limited supply of more complete proteins (7). The deficiency of total protein consumed is often the most pressing problem in many developing areas (12). The need of these areas can be met by increasing the protein content of the local grain staple through breeding improved varieties.

Physiological research should lead or parallel developments in breeding grain crops for protein content. Knowledge acquired about the mode of genetic expression of protein content would aid breeders and promote the attainment of high protein yields by production specialists. The information sought in this investigation was the role of the major nitrogen-assimilating systems in the production of grain protein.

REVIEW OF LITERATURE

It was commonly reported until 1954 that protein content of wheat and other grains varied little among varieties. Middleton, Bode, and Eayles (33) demonstrated that when domestic soft wheats were crossed with the selections 'Froncosa' and 'Fronteira' from Brazil, the protein content was increased as much as 3.2 per cent without an accompanying yield reduction. 'Atlas 50' and 'Atlas 66' were selected from such crosses. Subsequently, it has been shown that the increased concentration of protein is readily transmitted to adapted varieties (24, 48), and that the inheritance of protein content is controlled by few genes (49).

Differences in the grain protein content of corn from various hybrids were reported by Lang, Pendleton, and Dungan (29). Differences between 'Hy 2 x Oh 7' and 'Wf 9 x C 103' averaged over 2 per cent in favor of the latter variety. In contrast, soluble leaf protein content varied inversely between the two hybrids (16).

Studies in Illinois have also revealed variability in the protein content of soybeans. 'Amsoy' contained 2.5 per cent less seed protein than 'Wayne' over a 5-year period (3). Differences in protein content have also been reported among sorghum hybrids (Maranville, Personal Communication). An Israeli oat variety with a high protein content has been reported (Murphy, personal communication). Variability in the protein content of rice varieties has been observed at the International Rice Research Institute (27), and by the United States Department of Agriculture (Scott, personal communication).

General Nitrogen Relations

Differences in the nitrogen relations of high- and low- protein grain varieties have been sought to explain the biosynthesis of high protein contents. Seth, Hebert, and Middleton (47) reported that high protein content in wheat grain was not necessarily associated with high protein content in vegetative parts throughout the season. However, the high-protein wheat varieties increased more rapidly than low-protein varieties in protein content of the spike (47). This increase appeared to be due partly to increased translocation, since there was lower vegetative nitrogen content near maturity in the higher protein wheats (23). Stuber et al. (48) attributed the increase in grain protein of Atlas 66 over 'Wichita' partly to flowering date, because the variability in protein content of Atlas 66 showed it was more sensitive to adverse conditions during critical translocation. This lowered the starch: endosperm ratio, which raised the percentage value of protein (48). The same workers (Stuber et al.) reported that crosses between Atlas 66 and Wichita wheats produced individuals heterotically higher in grain protein than the high-protein parent. It was also observed that rust resistance and protein content were highly correlated, suggesting that a single gene controlled protein content (50). Thus, environmental effects on wheat protein content appeared to be supplementary. Johnson et al. (24) demonstrated that Atlas x 'Comanche' crosses produced additional protein with no decrease in yield, and that agronomic and quality characteristics exhibited sufficient variability to allow successful selection to improve those traits.

Work of Hageman et al. (17) with corn suggested that reduced nitrogen was the limiting factor in plant growth and that nitrate reductase limited nitrogen reduction. They showed that the protein content of leaves was positively correlated with nitrate reductase activity, which indicated that higher levels of nitrate reductase activity would increase protein synthesis and growth (16, 17). This supposition was supported by later trials correlating nitrate reductase activity, soluble leaf protein, and different nitrogen fractions (55). More alcohol-soluble nitrogen was incorporated into grain protein in the variety having more nitrate reductase activity (43). Further work with corn by Shrader et al. (42) on heritability of nitrate reductase activity among corn crosses showed heterotic effects in low x low crosses and inhibition of high x high crosses. The different effects made predicting and manipulating nitrate reductase activity of crops unrealistic.

Correlation of nitrate reductase activity in wheat to yield and protein production was studied by Croy (8). Nitrate reductase activity was influenced by factors which influenced the yield of grain and the yield of protein per acre in the varieties studied. Differences among varieties studied were slight for both nitrate reductase and grain protein (8).

Nitrate reductase has also been studied in soybeans, sorghum, and rice (2, 10, 35, 45, 50) and reviewed by Nason (36) and Nicholas (37). Despite the amount of work done, little evidence exists to suggest relationships between grain protein content and nitrate reductase activity in those species.

Glutamic acid dehydrogenase catalyzes production of glutamic acid from alpha-ketoglutaric acid and ammonia and is the main path of NH_3 incorporation in plants (5). The enzyme has been studied in plants (6, 9, 41), but not in

connection with accumulation of grain protein.

Glutamine, the second organic nitrogen compound formed in plants (47), is formed through the activity of glutamine synthetase, first isolated from plants by Elliot (11). Little work has been done linking activity of this enzyme to the level of grain protein production.

The transaminases catalyze amino acid synthesis from glutamic Acid and the alpha-keto precursors of the other amino acids. Glutamic-oxaloacetic transaminase is of particular importance in nitrogen assimilation (5, 18). Investigations into the role of these enzymes in the expression of grain protein content are obscure. However, glutamic-alanine transaminase activity was correlated with protein production in yeast (13).

Amino acid incorporation has been studied extensively with various techniques. The methods and results of these studies have been reviewed by Man (31), Schweet and Heintz (46), Webster (51), and others. Cell-free incorporation has been studied in both leaf organelles (1, 51) and grain endosperm components (14, 15, 52). Problems encountered in these studies include bacterial contamination (52), selective inhibition by substrate (1), and cofactor requirements. Intact tissue incorporation of amino acids has been studied (18, 25, 39), and the systems did not differ appreciably from ribosomal systems (31). These studies have provided insight into mechanisms and pathways of protein synthesis, but little information as to varietal differences in protein synthesis has been gained.

Protease activity in wheat seeds and seedlings has been reported to be higher in wheat varieties having a high grain protein content (40). This supported the idea that more complete translocation of nitrogenous compounds from vegetative parts is conducive to higher grain protein content (24).

MATERIALS AND METHODS

Experiment I

Wheat (Triticum aestivum L.), corn (Zea mays L.), sorghum (Sorghum bicolor(L.)Moench.), soybeans (Glycine max (L.) Merr.), oats (Avena sterilis L.), and rice (Oryza sativa L.) were selected from a number of lines of each species for marked differences in grain protein content. The low- and high-protein lines selected, their protein contents, and the authorities for the differences in protein contents are shown in Table I.

Seedlings of each line were germinated in moist vermiculite and transplanted to nutrient solutions 10 days after planting the rice and seven days after planting the other species. The nutrient solutions (Hoagland and Aron, 20) were in 2-liter containers and provided 10 mM KNO_3 , 5 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 1mM KH_2PO_4 , and 10 μM $FeNa_2EDTA$. Micronutrients were present at levels suggested by Johnson et al. (22). Rice received half-strength concentrations of the above nutrients plus 50 μM $MnSO_4$ and 200 μM SiO_2 (Okuda, 38; Yamasaki, 54). The nutrient solutions were maintained at pH 5.0 with HCl. Six plants of corn, sorghum, or soybeans or 12 plants of wheat, oats, or rice were held in each container. Eight replications of each line were randomized in complete blocks.

The seedlings were grown in environmental chambers maintained at 25c-15c day-night temperatures with a 16-hr light period and an 8-hr dark period. Lighting of about 1250 ft-c at plant height was provided by sixteen 160-watt fluorescent lamps and six 300-watt incandescent lamps. Relative humidity in the chambers was about 40 per cent. After two weeks growth, the seedlings were sampled for the assays described below.

Experiment II

Parents and low- and high-protein F_5 -progeny from crosses of low- and

Table I. High and low grain protein lines of wheat, corn, sorghum, soybeans, oats, and rice studied for moieties associated with protein content.

Species	Line	Protein Content	Reference
---	---	%	---
Wheat	Atlas 66	18.8	Middleton et al. (1954),
	Pawnee	15.7	Heyne (Personal Comm.)
Corn	WF9 x C103	11.3	Lang et al. (1956)
	Hy2 x Oh7	8.8	
Sorghum	C.I. 3071	17.8	Maranville (Personal Comm.)
	Plainsman	10.2	
Soybeans	Wayne	41.1	Bernard et al. (1967)
	Amsoy	38.5	
Oats	C.I. 8330	25.1	Murphy (Personal Comm.)
	Animated Oats	11.3	
Rice	Taichung Native #1	11.1	Juliano et al. (1964),
	Bluebelle	7.1	Scott (Personal Comm.)

high-protein wheat varieties were studied to determine inheritance of factors associated with grain protein contents. The parents, progeny, and their respective protein contents were 'Kaw' (13.7%), 'Triumph' (12.3%), Atlas 50 (18.4%), Atlas 66 (18.8%), Kaw x Atlas 50 (13.0 & 18.0), Kaw x Atlas 66 (17.8 and 18.0%), Triumph x Atlas 50 (13.7 and 18.7%), and Triumph x Atlas 66 (14.0 and 18.4%) (Heyne, personal communication). Four replications of the seedlings were germinated and grown as described above for wheat and sampled after two weeks for the assays described below.

Experiment III

'Pawnee' and Atlas 66 wheats were seeded at the rate of 101 kg per ha in 3-m x 8-m plots October 18, 1967, on the Kansas State University Agronomy Farm, Manhattan, Kansas. Each plot was replicated four times in a completely randomized design. The plots were topdressed with 200 kg per ha of N as NH_4NO_3 on March 30, 1968. Plants were sampled randomly from each plot at weekly or biweekly intervals from April 2, 1968, to July 2, 1968, for the assays described below. The date and stage of maturity of the wheat at each sampling are shown in Table II.

Sampling and Assays

Leaf material removed from the plants was cut into 1-cm sections. Grain for assays was threshed from the heads from field-grown plants. A 1-g subsample of leaf or grain tissue was homogenized in 20 ml of medium (33 mM Tris, 3.3 mM cysteine, and 0.1 mM Na_2EDTA adjusted to pH 7.2 with HCl) for 1 min with a Servall Omnimixer at 16,000 rpm with the cup immersed in an ice bath. Unmacerated tissue was restored to the blending medium and the contents were homogenized for another minute. The homogenate was filtered through a fine-mesh sieve and centrifuged at 20,000 x g for 15 min at 2°C.

Table II. Dates Pawnee and Atlas 66 attained the various spring growth stages.

STAGE	DATE	
-----	Pawnee	Atlas 66
Tillering	April 2	April 2
Jointing	April 30	May 14
Heading	May 14	May 28
Anthesis	May 21	June 4
Dough	June 4	June 18
Mature	June 25	July 9

The supernatant was filtered through glass wool and held at 2-3C in an ice bath. The enzyme activity assays were performed at 30 C for 20 min, with the exception of the wheat in Experiment II, which was incubated for 30 min for the nitrate reductase assay.

The method of Hageman and Flesher (16) was employed for assay of nitrate reductase activity. Reaction mixtures consisted of 1 ml of 0.2 \underline{M} KH_2PO_4 (pH 7.2), 0.2 ml of 0.1 \underline{M} KNO_3 , 0.5 ml of 1.3 \underline{mM} NADH, and 0.3 or 0.5 ml of enzyme extract. The reaction was stopped with 1 ml of 1% sulfanilic acid (w/v) in 1.5 \underline{N} HCl and color was developed with 1 ml of 0.02% (w/v) N-(1-naphthyl) ethylene diamine dihydrochloride. Absorbance was measured at 540 mu against a reagent blank in which extraction medium replaced enzyme. Absorbance of the sample blank, in which phosphate buffer replaced NADH, was subtracted from sample readings.

Glutamic dehydrogenase activity was determined by the method of Lowry et al. (30). Harper (18) showed 95% of the enzyme activity was recovered in the supernatant extract of wheat, although the enzyme has been reported in mitochondria (Ritenour et al., 41). Assay mixtures consisted of 1 ml of 2.0 \underline{M} KH_2PO_4 (pH 7.2), 0.5 ml of 0.1 \underline{M} $(\text{NH}_4)_2\text{SO}_4$, 0.5 ml of 1.3 \underline{mM} NADH, and 0.5 ml of enzyme extract. A 0.1-ml aliquot of mixture was placed in 10 ml of 5 \underline{N} NaOH and fluorescence was measured on a Turner Model 110 Fluorometer using a 7-60 primary filter (maximum transmittance at 365 mu) and a 75 secondary filter (maximum transmittance at 485 mu). The instrument was zeroed with a reagent blank in which extraction medium replaced the extract.

The method of Elliot (11) was used for determining glutamine synthetase activity. The reaction mixture consisted of 1 ml of 0.8 \underline{M} Tris, 1 ml of 0.5 \underline{M} sodium glutamate (both at pH 7.2), 0.2 ml of 1 \underline{M} MgSO_4 , 0.2 ml of 1 \underline{M} hydroxylamine, 1 ml of 0.025 \underline{M} ATP (adenosine triphosphate), and 1 ml of

enzyme extract. Ferric chloride reagent (equal volumes of 10% (w/v) $\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$ in 0.2 M HCl, 24% (w/v) TCA (trichloroacetic acid), and 50% (w/v) HCl was added to develop color. The contents of the tubes were centrifuged at 1500 x g for 5 min and absorbance was determined at 540 mu against a reagent blank in which reaction medium replaced the enzyme. The absorbance of sample blanks, prepared by substituting water for ATP, was subtracted from that of the corresponding sample.

The method of Karmen et al. (27) was used for the assay of glutamic-oxaloacetic transaminase activity. One ml of enzyme extract was added to 1 ml of substrate solution (0.02 M aspartate and 0.02 M alpha-ketoglutarate in 0.2 M KH_2PO_4 at pH 7.2). Color reagent (1 mg dinitrophenylhydrazine/ml of 1.35 M HCl) was added to stop the reaction. Color was developed at 30C for 10 min and 10 ml of 0.4 M NaOH was added. The contents were diluted with 10 ml of water and absorbance was measured at 504 mu against a reagent blank in which extraction medium replaced the enzyme extract.

Enzyme extracts and protein synthesis extracts were analyzed for soluble protein content by the modified Folin technique of Miller (34). One ml of alkaline copper reagent was added to the protein solution and allowed to react for 10 min at room temperature. Three ml of diluted Folin reagent were added rapidly and the contents of the tubes were heated for 10 min at 50 C. After cooling, the samples were read at 650 mu against a reagent blank in which water replaced the protein solution.

Free amino acid content of the field wheat samples was determined by the method of Rosen (42). One ml of diluted extract was mixed with 1 ml of ninhydrin reagent and the samples were heated at 100 C for 15 min. After cooling, 5 ml of color diluent were added to each sample and absorbance was read at 570 mu against a blank in which water was substituted for the extract.

Nitrate content of the extracts was measured periodically to determine if nitrate uptake differed among the species. This was done by the procedure of Wooley, Hicks, and Hageman (53). Ten ml of 20% acetic acid containing 0.2 ppm CuSO_4 were added to 2 ml of the diluted extract. Approximately 0.4 g of reducing powder was added to each sample and the contents were shaken vigorously for 15 sec every 3 min for a total of three times. The suspensions were centrifuged at 6,000 x g for 5 min and the supernatant was filtered through glass wool. Absorbance was measured at 520 mu against blanks in which water replaced extract solution.

Protein synthesis was determined by the method of Key (28). One g of sectioned leaf blades or grain was preincubated for 2 hr at 2 C in 1% sucrose solution. The samples were blotted and placed in incubation medium (4 ml of $6.24 \times 10^{-3} \text{M}$ phosphate buffer, pH 6.0 containing 1.25% (w/v) sucrose and 80 ug streptomycin, and 1 ml of C^{14} -L-leucine with an activity of 0.25 uc). The medium was vacuum infiltrated into the samples which were incubated for 2 hr at 30 C with constant shaking. The medium was decanted and the tissue was frozen at -20 C until extraction. The tissue was extracted in 20 ml of ice-cold 0.1 M Tris buffer (pH 7.5) containing 4 mg of C^{12} -L-leucine. Homogenization was performed as with the enzyme samples. The homogenate was filtered through glass wool and protein was precipitated from a 15-ml aliquot with 15 ml of 10% (w/v) TCA. The protein pellet formed after centrifugation at 5,000 x g for 10 min was dissolved in 1 ml of 3 M NaOH containing 0.2 mg of C^{12} -L-leucine. Whatman No. 3 filter paper discs 2.3 cm in diameter were spotted in duplicate with 0.1-ml aliquots of the protein solution (Mans and Novelli, 32). The discs were placed in ice-cold 10% TCA containing 0.1 M C^{12} -L-leucine for 1 hr with occasional swirling and washed 15 min with TCA of the same volume and concentration. The discs were suspended in 1:1 (v/v)

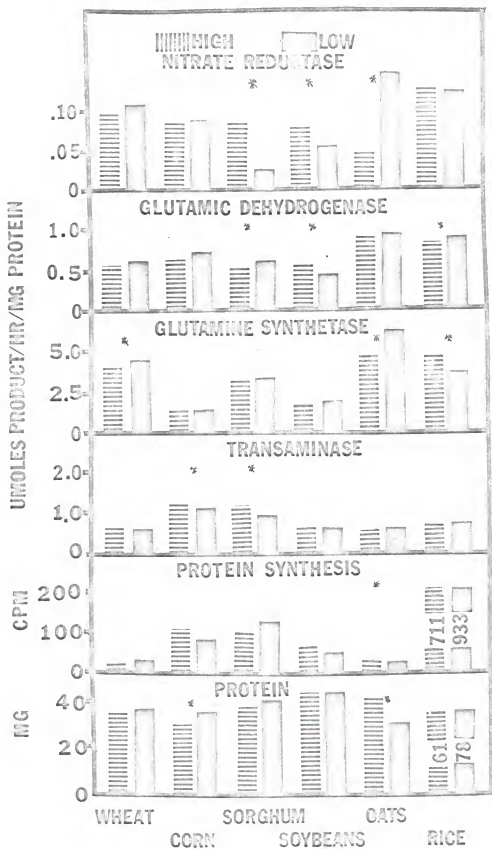
ether-ethanol for 30 min at 37 C. The mixture was replaced with ether for 15 min and the discs were air-dried. For counting, the discs were placed in low-potassium glass counting vials containing 5 ml of scintillation mixture (0.4% (w/v) 2,5-diphenyloxazole (PPO), and 0.01% (w/v) p-bis-2(2-(4-methyl-5-phenyloxazolyl)- benzene (dimethyl POPOP) in absolute toluene). The samples were counted twice each for 100 min on a Packard Model 314 EX Tri-Carb liquid scintillation spectrometer.

RESULTS

Experiment I

The results from Experiment I are given in Figure I. Nitrate reductase activity was higher in the high-protein variety than in the low-protein variety for sorghum, soybeans, and rice. The difference between varieties of sorghum and soybeans was significant at the 5% level. Activity was greater in the low-protein variety than in the high-protein variety in wheat, corn and oats. The difference was significant only in oats, however. Glutamic dehydrogenase activity was significantly greater only in the high-protein soybean variety. All the other species contained higher glutamic dehydrogenase activity in the low-protein lines than in the high-protein lines. The advantage of the low-protein variety was significant in sorghum and rice. Glutamine synthetase activity was greater in the high-protein than in the low-protein variety of soybeans and rice, but only in the latter species was the difference significant. The low-protein varieties of wheat and oats contained significantly more glutamine synthetase activity than the high-protein varieties, while other species differed little between varieties. Glutamic-oxaloacetic transaminase activity was significantly greater in the high-protein variety of corn and sorghum. The high-protein wheat variety showed the same trend. Transaminase activity in the other species differed little between varieties. Protein synthesis by intact leaf tissue was significantly greater in the high-protein oats variety. High-protein corn and soybeans also had higher protein synthesis rates, but the differences were not significant. The other species showed the opposite trend, though none of the differences were significant. Soluble leaf protein content was significantly greater in the high-protein variety only in oats. All the other species contained more soluble protein

Factors studied in seedlings of low- and high-protein varieties of six crop species listed in Table I. *Varieties differed at 5% level.

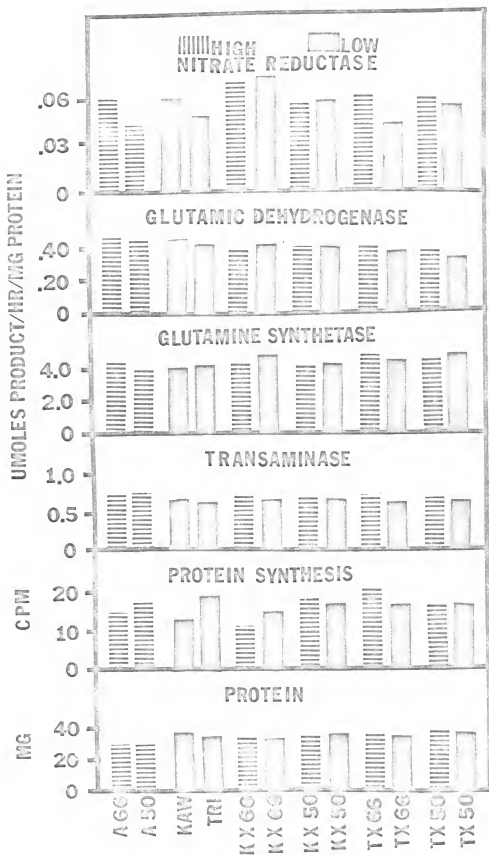


in the low-protein lines, but only the difference in corn was significant.

Experiment II

The results of the experiment with wheat parents and their progeny are presented in Figure 2. In the parents, nitrate reductase activity was greater in Kaw than in Atlas 66, and both of those were greater than in Triumph and Atlas 50. The progeny showed no overall differences between low- and high-protein selections. When the two parents highest in nitrate reductase activity were crossed, heterotically higher levels of activity were obtained in both selections. Glutamic dehydrogenase activity was slightly higher in high-protein parents and selections than in the low-protein parents and selections, but the differences were not significant. Glutamine synthetase activity of the parents was significantly greater in Atlas 66 and Triumph than in Kaw and Atlas 50. In the progeny, no overall difference was found between the high- and low-protein selections, but the low-protein selections tended to have more activity than the high-protein selections. Glutamic-oxaloacetic transaminase activity was significantly greater in Atlas 50 than in the other parents, and Atlas 66 tended to have more activity than Kaw and Triumph. The high-protein selections of the progeny contained significantly greater transaminase activity than the low-protein selections. Protein synthesis activity in the parents did not differ significantly. The order of decreasing protein synthesis activity was: Triumph, Atlas 50, Atlas 66, and Kaw. No overall difference between high- and low-protein selections was found in the progeny. Soluble leaf protein content was significantly greater in Kaw than in Triumph, Atlas 50, or Atlas 66. The soluble leaf protein content of the parents ranked in inverse order to the grain protein content. In the progeny, however, there was no overall difference in soluble leaf protein content between the high- and low-protein selections.

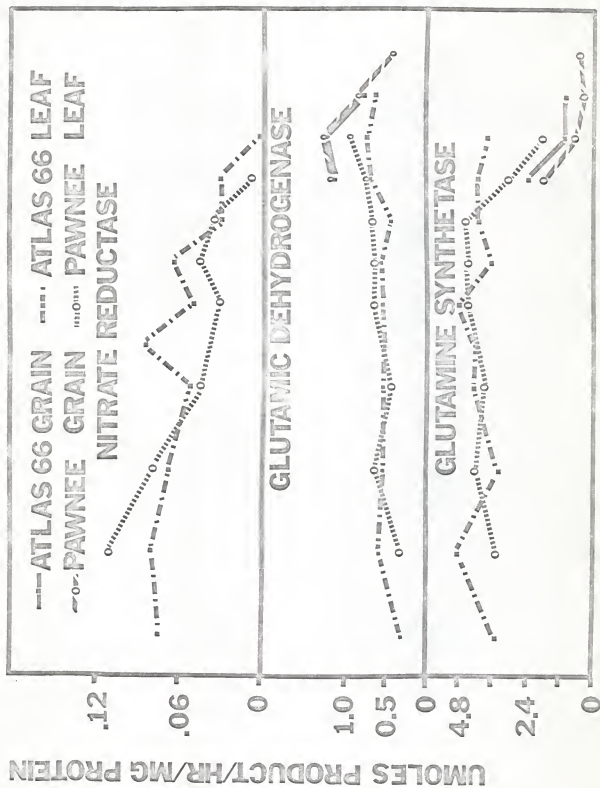
Factors studied in seedlings of low- and high-
protein wheat varieties and their F_5 high- and
low-protein progeny.



Experiment III

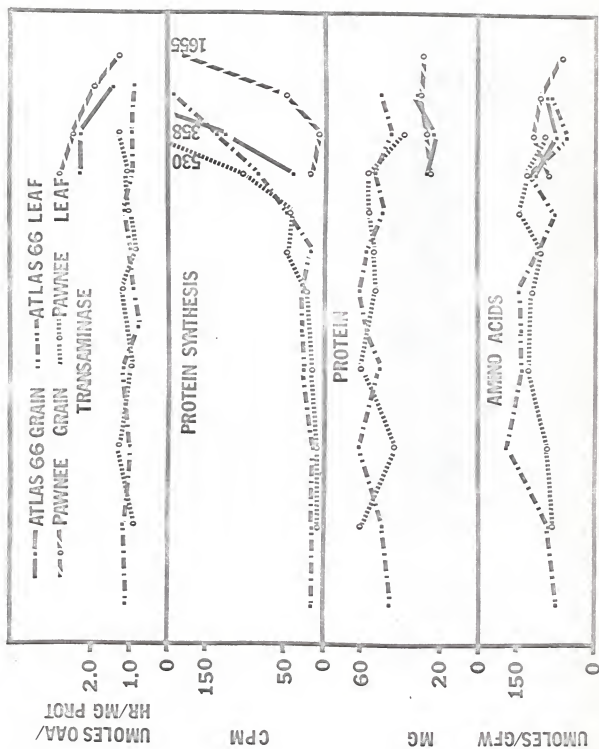
Atlas 66 and Pawnee data are shown in Figures 3 and 4. Nitrate reductase activity in the leaf tissue of both varieties was relatively high early during the spring and gradually declined during maturation. Nitrate reductase activity was greater in Pawnee than in Atlas 66 during early maturity stages. These results agreed with those from the environmental chamber experiment comparing the two varieties. Atlas 66 surpassed Pawnee in nitrate reductase activity at later stages and maintained activity for a longer period at the end of the growing season. Nitrate reductase activity in the grain was low and did not appear to contribute significantly to nitrogen metabolism in the spike. Glutamic dehydrogenase activity in the leaves gradually increased during the spring growth period. Activity in the grain also increased during maturation. Trends of glutamic acid dehydrogenase activity were quite similar in the two varieties in both leaves and grain, so no differences were detected. Glutamine synthetase activity in both leaves and grain declined during spring growth. Activity was initially higher in the leaves of Atlas 66 than in Pawnee, as was true in Experiment I. The difference between the two varieties later disappeared, but glutamine synthetase activity was maintained at a constant level longer in Atlas 66 than in Pawnee. Activity in the grain was generally greater in Atlas 66 than in Pawnee. Glutamic-oxaloacetic transaminase activity in leaves did not differ markedly between the two varieties during the season, unlike the environmental chamber results. Pawnee, however, tended to have higher transaminase activity in the grain than Atlas 66. Protein synthesis activity was constant in both varieties until early senescence, when both leaf and grain protein synthesis activity appeared to increase markedly: Differences between the varieties were slight until this period of rapid increase. Protein synthesis in the leaves of

Nitrate reductase, glutamic acid dehydrogenase, and glutamine synthetase activities in leaves and grain of Atlas 66 and Pawnee wheat during the spring growth cycle. Sampling dates were adjusted for differing maturities as shown in Table II.



TILLERING JOINTING HEADING ANTHESIS DOUGH

Transaminase and protein synthesis activities and soluble protein and free amino acid contents in leaves and grain of Atlas 66 and Pawnee wheat during the spring growth cycle. Sampling dates were adjusted for differing maturities as shown in Table II.



TILLERING JOINTING HEADING ANTHESIS DOUGH

Pawnee increased at a faster rate and to a higher value than in Atlas 66. Protein synthesis by the grain of Atlas 66 initially increased at a greater rate than Pawnee, but Pawnee grain achieved an extremely high rate of synthesis at maturity. Soluble protein content of leaf and grain tissue was generally constant during the spring season, although cyclic fluctuations were evident. The two varieties were similar in soluble protein content for most of the season, however, and the persistence of a high level of soluble protein in leaves of Atlas 66 near maturity can be attributed partly to higher nitrate reductase and glutamine synthetase activity during that stage. Free amino acid content of both leaf and grain tissue was initially greater in Atlas 66 than in Pawnee, but the difference later disappeared.

DISCUSSION

No single factor studied in the leaves of the six grain species was consistently associated with grain protein content. Nitrate reductase activity of wheat was generally higher in the low-protein variety than in the high-protein variety. Similarly, the low-protein wheat variety had significantly more glutamine synthetase activity. Only transaminase activity tended to correspond to grain protein content. Croy (8) demonstrated that nitrate reductase activity in wheat corresponded to grain protein production, but the increase obtained was related to grain yield rather than to grain protein concentration. Enzyme activities expressed on the basis of whole-plant activity would probably show activities were greater in Atlas 66 than in Pawnee, because the former produced more vegetative growth. However, specific activity was chosen as a better indicator of genetic potential so that the degree of foliage accumulation wouldn't affect the results. The results of Seth et al. (47) and Johnson et al. (23) are reflected in the soluble leaf protein content of the two varieties in that the vegetative parts contained less nitrogen in the high-protein variety than in the low-protein variety of wheat.

Transaminase activity in corn leaves was a better indicator of protein than the other factors studied. Nitrate reductase activity obtained generally agreed with reports by Hageman et al. (16, 17) and Zieserl et al. (55) with the same two varieties. However, the designations of high- and low-protein differed according to whether leaves or grain was measured. The designation used here was according to grain protein content (29). The same papers (16, 17, 55) can be cited in support of soluble leaf protein data from the corn varieties. All these reports state that the low-grain protein variety

used here was higher in soluble protein content of the shoots than was the high-grain protein variety used.

Nitrate reductase and transaminase activities in sorghum corresponded well to grain protein content. The high-protein line was significantly higher in activity of both enzymes than the low-protein line. Cyanide inhibition of nitrate reductase activity undoubtedly decreased the in vitro values from the actual level of activity. However, barring a pronounced difference in cyanide production between the varieties, the activity measured should be indicative of the actual activity of each variety. Soluble protein content varied inversely so that the low-protein line was higher than the high-protein line.

Both nitrate reductase and glutamic dehydrogenase activity in soybeans corresponded to grain protein content. There was little difference between the varieties in the other factors studied. Unlike other species, soybeans contain appreciable nitrate reductase activity in the particulate fraction of leaf extracts (Harper, personal communication). The significance of this particulate nitrate reductase activity is not known. However, since sedimentation rates of the organelles of the two varieties should be similar, the same relative activity should have been obtained in the cytoplasm of both varieties.

Soluble leaf protein content and protein synthesis were associated most closely with the grain protein content in oats. The low-protein line contained significantly more nitrate reductase and glutamine synthetase activities than the high-protein line. The difference between the lines was magnified by expressing the enzymes as specific activity. Although oats was the exception in more than one of the attributes studied, it should be noted that those difference represented the broadest genetic variation of

any species studied.

Both varieties of rice exhibited high nitrate reductase and protein synthesis activities and high soluble protein contents. The last attribute can be explained by the high dry matter percentage of rice as compared with the other species since the soluble protein was expressed on a fresh weight basis. The dryness of the tissue was due to nitrogen deficiency, since rice cannot assimilate nitrate efficiently (4). This would also explain the high protein synthesis rate. Harper (18) obtained high protein synthesis rates by wheat plants severely deficient in nitrogen, because of the lack of a free amino acid pool to inhibit exogenous amino acid incorporation. The high nitrate reductase activity obtained from rice, a species which cannot assimilate nitrate, is still unexplained, however. Perhaps another enzyme functioning in nitrate reduction may be absent or low in activity in rice. Glutamic dehydrogenase and glutamine synthetase activities differed significantly between the low- and high-protein rice varieties. Glutamine synthetase activity was greater in the high-protein variety, reflecting the trend in nitrate reductase activity. Glutamic dehydrogenase activity, however, was greater in low-protein variety than in the high-protein variety. Protein synthesis and soluble protein content were also greater in the low-protein variety, but the differences were not significant. The best indicator of grain protein content of the factors studied in rice was glutamine synthetase activity.

Nitrate reductase activity in the leaf blades of young wheat seedlings followed no pattern relative to grain protein content in the four parent varieties. The greater difference among progeny than between selections of the progeny suggested that the two factors were not closely linked. The heterosis

of nitrate reductase activity obtained in the high x high cross (with respect to nitrate reductase activity) differed from the report of the heritability studies of Schrader et al. (44) with corn. That report stated that no high x high cross exceeded the midparent level of nitrate reductase activity. There appeared to be little genetic variation in glutamic dehydrogenase or glutamine synthetase activity. Transaminase activity correlated better with grain protein content than any of the other factors. The differences were small but consistent, indicating that a more sensitive assay should be sought. Differences in protein synthesis rates among the parents and progeny were insignificant. Kaw contained more soluble leaf protein than the other parent varieties. The observation that low-protein parents were higher in soluble protein than the high-protein parents related well with Experiment I, but soluble protein contents within and among the progeny of the crosses were similar.

Nitrate reductase activity during the spring growth period of winter wheat demonstrated the value of season-long activity determinations as used by Schrader and Hageman with corn (43). The area under the curve of nitrate reductase activity obtained for Atlas 66 was more than one and one-half times greater than the area under the nitrate reductase activity curve obtained for Pawnee. Thus, Atlas 66 had more nitrate reductase activity for a longer time than did Pawnee. The importance of timing is evident if nitrate reductase activity is used to predict grain protein content. The general decline of nitrate reductase activity in both varieties reflected the decline in the photosynthetic ability of the tissue. The same trend was shown by Harper and Paulsen (19) and by Croy (8) in wheat. The gradual increase of glutamic dehydrogenase activity over the season in leaves of both varieties may have

been due to an increased amount of ammonia substrate available from degradation of proteins. The maintenance of glutamine synthetase activity in leaves of Atlas 66 was probably related to the higher amount of nitrate reductase activity at the corresponding growth stage, since those two enzymes appear to be closely related in wheat (18). The glutamine synthetase activity in both varieties reflected the downward trend in nitrate reductase activity because of this relationship. The varieties were similar in transaminase activity throughout the season. Constant levels of activity were maintained throughout the sampling period in leaf tissue. Activity in the grain, however, declined at maturity as substrate probably became limiting. Protein synthesis activity in the leaves of the high-protein variety increased more slowly during spring development and continued longer than in the low-protein variety. Protein synthesis by the grain initially showed a faster rate of increase in the high-protein variety. The high amount of incorporation by the dry, senescent tissue of both varieties was similar to the results of Harper (18) in nitrogen-deficient wheat. The lack of a free amino acid pool to inhibit C^{14} -leucine incorporation led to the high activity obtained (1). The earlier, gradual increase in protein synthesis by both varieties may have been related to an increase in energy-rich compounds from degradation. The fluctuation of soluble protein of both leaf and grain tissue coincided when the same sampling dates were compared, which indicated an influence from environmental factors. The fact that soluble protein content was more constant between varieties than among sampling dates suggested that a similar balance of nitrogen metabolites was present in both varieties. The overall picture of nitrogen metabolism presented by the free amino acid content data is related to rates of amino acid synthesis, protein break-down, and protein synthesis (21, 40). The curves obtained for the two varieties reflected the

interrelationships of those three factors. The greater amount of free amino acids in the leaves of Atlas 66 at early stages was apparently stored in forms other than soluble leaf protein, as the amount decreased to below the level present in Pawnee without an accompanying increase in soluble protein. The two varieties contained similar levels of free amino acids at maturity. The same trends were apparent in free amino acids in the grain, although the varieties did not differ. Decline of the free amino acid content was not accompanied by an increase in the soluble protein content.

SUMMARY

Low- and high-protein varieties of wheat (Triticum aestivum L.), corn (Zea mays L.), soybeans (Glycine max (L.) Merr.), sorghum (Sorghum bicolor (L.) Moench.), rice (Oryza sativa L.), and oats (Avena sterilis L.) did not differ consistently in nitrate reductase activity, glutamic dehydrogenase activity, glutamine synthetase activity, glutamic-oxaloacetic transaminase activity, protein synthesis by intact leaf tissue, or soluble leaf protein content. However, nitrate reductase and transaminase activities and protein synthesis were usually higher in the high-protein lines. Transaminase activity was most consistently and protein synthesis least consistently associated with grain protein content. Glutamic dehydrogenase and glutamine synthetase activities and soluble leaf protein content were usually higher in the low-protein lines. Soluble leaf protein content was most consistently inversely associated with grain protein content. Notable exceptions to these generalities were present in all the attributes except transaminase activity.

Experiment II more closely examined relationships among seedlings of two high-protein wheat varieties (Atlas 66 and Atlas 50) and two low-protein varieties (Kaw and Triumph) and the F₅ high- and low-protein progeny. No differences existed between the low- and high-protein lines in nitrate reductase, glutamic dehydrogenase, or glutamine synthetase activities or in protein synthesis and soluble leaf protein content. Transaminase activity, however, was greater in the high-protein selections than in the low-protein selections.

Experiment III attempted to relate enzymatic activities and protein content during spring growth of winter wheat. Similar growth stages were compared in Atlas 66, the high-protein variety, and Pawnee, the low-protein variety. Nitrate reductase and glutamine synthetase activities in the leaf blades

declined during the sampling period, but at later stages Atlas 66 maintained higher rates of activity of the two enzymes. Nitrate reductase activity in the grain was negligible, but glutamine synthetase activity in the grain was higher in Atlas 66 than Pawnee. Both enzymes, however, declined with maturity. Glutamic acid dehydrogenase activity in the leaves increased slightly with maturity, while activity in the grain declined. Similar levels of activity were present in the leaves and grain of the two varieties. Transaminase activity in the leaves was constant over the sampling period, while activity in the grain declined with maturity. The only difference between the varieties occurred in the grain, with Pawnee slightly higher in activity than Atlas 66. Protein synthesis was constant early in the season, but markedly increased near maturity in both leaves and grain. The increase in protein synthesis in the leaves and grain was greater in Pawnee than in Atlas 66, but Atlas 66 initiated a high level of synthesis earlier than Pawnee. Soluble protein content in leaves and grain was constant over the season and between varieties. Free amino acid content did not change markedly over the season. Atlas 66 was initially higher in amino acid content than Pawnee, but the difference later disappeared.

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NITROGEN ASSIMILATION AND PROTEIN SYNTHESIS IN LOW- AND
HIGH-PROTEIN LINES OF WHEAT AND OTHER GRAIN SPECIES

by

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Low- and high-protein lines of wheat (Triticum aestivum L.), corn (Zea mays L.), soybeans (Glycine max (L) Morr.), sorghum (Sorghum bicolor (L.) Moench.), rice (Oryza sativa L.), and oats (Avona sterilis L.) were studied for possible relationships among nitrogen assimilation, protein synthesis, and grain protein content. In leaves of two-week-old seedlings grown under controlled conditions, nitrate reductase, transaminase, and protein synthesis activities were usually higher in the high-protein lines. Transaminase activity was most consistently and protein synthesis least consistently associated with grain protein content. Glutamic dehydrogenase and glutamine synthetase activities and soluble leaf protein content were usually higher in the low-protein lines. Soluble leaf protein content was most consistently inversely associated with grain protein content. Notable exceptions were present in all attributes studied except transaminase activity.

Seedlings of two high-protein wheat varieties ('Atlas 66' and 'Atlas 50') and two low-protein varieties ('Kaw' and 'Triumph') and their F_3 progeny varied little in enzymatic activities. Transaminase activity, however, tended to be higher in the high-protein parents than in the low-protein parents, and was significantly higher in the high-protein progeny than in the low-protein progeny. Glutamine synthetase activity also differed significantly, but not in relation to protein content.

Low-protein 'Pawnee' and high-protein Atlas 66 wheat grown in the field had similar trends of enzymatic activities during the spring growth cycle. Levels and duration of some enzymes differed, however. Nitrate reductase and glutamine synthetase activity in the leaves were higher and persisted longer in Atlas 66 than in Pawnee. Protein synthesis in the leaves near senescence was greater in Pawnee than in Atlas 66. Other factors studied

in the leaves did not differ. In grain tissue, glutamine synthetase activity and the initial rate of protein synthesis were higher in Atlas 66 than in Pawnee, and glutamic dehydrogenase and transaminase activities were higher in Pawnee than in Atlas 66.