EFFECT OF NITROGEN, PHOSPHORUS AND POTASSIUM ON WHEAT (*TRITICUM AESTIVUM* L.) GRAIN PROTEIN

by

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B.S., University of Minnesota, 1975

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Agronomy

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1979

Approved by:

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ACKNOWLEDGEMENTS

I wish to thank my major professor, Dr. Gary Paulsen, for advice and encouragement throughout my research and manuscript preparation.

I wish also to acknowledge the contributions of Dr. David Kissel and Dr. Lawrence Davis in stimulating and clarifying my thoughts on this topic.

Finally, I wish to acknowledge the substantial contributions of my wife, Sandra, in both the writing of this thesis and the work in preparing the research.

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REVIEW OF LITERATURE

The path of nitrogen from the soil to grain protein has been extensively studied. Nitrate is taken up by the roots (Wallace and Pate, 1965) and reduced in leaves by nitrate reductase (NR) and nitrite reductase enzymes to ammonia, which is assimilated into organic forms (Lea and Miflen, 1974). Reductive aminations, transaminations and transformations (Fowden, 1967; Miflen and Lea, 1976) provide all the amino acids for protein synthesis.

It has been suggested that NR is the controlling factor in nitrogen assimilation (Filner, 1966; Beevers and Hageman, 1969). Experiments showed that grain protein percentage was related to leaf NR activity within a variety and that there is probably a characteristic genetic relationship between NR activity and grain protein (Croy, 1967; Croy and Hageman, 1970). However, an earlier study (Seth et al., 1960) showed no differences in protein content of the vegatative plant parts, between high and low protein wheats, at any stage of development. They concluded that other factors besides nitrogen assimilation controlled grain protein deposition. Other studies (Zierserl et al., 1963) showed no clear relationship between NR activity and maize protein concentrations. Soybean studies (Adjei-twum and Splitt-stoesser, 1976) indicated that nitrogen content and NR activity per gram-fresh weight determined seed protein percentage while nitrogen content and NR activity per whole plant determined total seed protein.

Nitrate reductase (E.C. 1.6.6.1) is a molybdoflavoprotein which utilizes $FMNH_2$ or NAD(P)H to reduce nitrate to ammonium (Beevers and Hageman, 1969). Many factors including light, drought stress, genotype, tissue age and organ type control gross NR activity (Beevers and Hageman, 1969). Fine control of NR activity may be through induction by nitrate (Afridi and Hewitt, 1964), nitrate flux (Shaner and Boyer, 1976), phytochrome interactions (Filner and Klein, 1968; Jones and Sheard, 1972), ADP inhibition (Eaglesham and Hewitt, 1971; Nelson and Ilan, 1969), endogenous amino acid level (Filner, 1966; Joy, 1969; Oaks et al, 1977; Radin, 1977) and secondary metabolite regulation (Schrader and Hageman, 1967). Phosphate deficiency inhibited NR activity (Pirson, 1955) while phosphate activated NR in in vitro assays (Ferrari and Verner, 1970; Kinsky and McElroy, 1958). Potassium has not been shown to have any direct effect on NR activity.

The effect of nitrogen (N), phosphorus (P) and potassium (K) fertilization on leaf and grain protein has been studied (Mosolov and Volliedt, 1962). High N:P fertilizer ratios decreased leaf protein and leaf nitrogen concentrations. With N:P ratios slightly greater than 1.0, both leaf protein and carbohydrate concentrations were high. With low N:P ratios, both leaf protein and carbohydrate concentrations were low. Grain protein percentages, in the same experiments, decreased as P fertilization increased except at the highest P treatment. Increasing K fertilization increased grain protein, leaf reducing sugar and total leaf carbohydrate concentrations.

Interactions among N, P, and K uptake rates have been noted. Nitrogenous fertilizers increased P uptake by corn (Cole et al., 1963).

P uptake was stimulated more by N pretreatment than by a ten-fold increase in external P (Cole et al., 1963)

Total plant nitrogen decreased with increasing P on low N, low K soils (Sorenson, 1971). Moderate P increased plant nitrogen concentration. At very low N fertilization, P enhanced NR activity but did not change protein synthesis rates. With high N and P fertilization, nitrogen distribution shifted to a relative increase in protein synthesis (Sorenson, 1971). Phosphorus fertilization of spring wheat grown on 1.0 p.p.m. P test soil had no effect on grain nitrogen content or test weight and no N-P interaction was noted (Gardener and Jackson, 1976).

Low K fertilization limited nitrate uptake and transport in barley (Blevins et al., 1978; Frost et al., 1978). Xylem exudates had approximately equal concentrations of potassium and nitrate when plants were pretreated with K, but calcium and sodium pretreatments resulted in four times as much nitrate as potassium (Frost et al., 1978). On the basis of these lines of evidence, it was proposed that K acts as a counterion in nitrate uptake and transport (Lips et al., 1971). In this model, the K moves into the shoot as the nitrate counterion and then returns to the root as a counterion of malate or some other organic acid. The organic acid is degraded metabolically to provide bicarbonate for exchange in the soil solution (Lips et al., 1971). Results involving K stimulation of nitrogen metabolism have been inconsistent. In corn, K deficiency led to high leaf protein concentration but lower total protein per shoot. It was postulated that K stimulated peptide synthesis but that low growth rates limited total protein production (Hsiao et al., 1970). The role

of K as a counterion has been disputed (Kirkby and Knight, 1977). They suggested that K acts as a counterion during uptake and transport, but is sequestered in the shoot vacuole. Currently, the weight of evidence favors a strong corellation between K fertilization and nitrate uptake and transport.

K deprivation stimulated P uptake in corn (Classen and Barber, 1977) indicating that secondary interactions might be found. In such a system, K deprivation would cause a series of metabolic alterations which would result in changes in nitrate and protein metabolism.

Field application of N, P and K fertilizers and mixed results on wheat grain protein. Some N fertilization studies showed no grain protein effect while others showed significant protein increases (Murphy and Gallagher, 1976; Murphy et al., 1977). Phosphorus application had no effect on grain protein in most experiments (Leikem et al., 1978; Murphy and Gallagher, 1976; Murphy et al., 1977) though significant declines in grain protein were sometimes noted (Murphy et al., 1977) at higher P application rates. K fertilizer trials showed no effect on grain protein (Lundquist and Murphy, 1978; Murphy et al., 1977). These studies were preformed on high K soils. Earlier studies which did show an effect were cited.

Wheat grain yield and grain protein concentration have been assumed to have a inverse relationship. This is probably based more on environmental conditions than on genetic potential (Johnson and Mattern, 1976). Several high-yield high-protein varieties have recently been developed (Goertzen and Goertzen, 1976; Johnson and Mattern, 1976). Certain of these varieties have shown a strong dependence on high soil fer-

tility (Goertzen and Goertzen, 1976). At low soil fertility, protein production may be sacrificed to maintain yield.

We determined if a representative high-protein variety, "Plainsman V", showed a differential response in protein metabolism with varying fertilizer levels. Leaf NR activity, soluble protein and soluble carbohydrate were considered appropriate indicators of metabolic response.

MATERIALS AND METHODS

Hydroponic Studies

Seeds of 'Plainsman V' wheat (Triticum aestivum L.) were germinated in vermiculite with distilled, deionized water. Seedlings were grown in constant light at 25 C for two weeks and then transplanted to pots containing nutrient solutions modified from the basic Hoagland's medium (Hoagland and Arnon, 1950). Calcium was supplied as the chloride while magnesium, manganese, zinc and copper were supplied as sulfates. Boron was supplied as boric acid and molybdenum was supplied as molybdic acid, all in the concentrations recommended. Nitrogen was supplied as sodium nitrate at three levels; 5 mN, 10 mM, and 15 mM. Potassium was supplied as the chloride at three levels; 0.05 mM, 0.5 mM and 5.0 mM. Phosphorus was supplied as sodium phosphate (pH 5.0) at five levels; 0.005 mM, 0.01 mM, 0.05 mM, 0.1 mM, and 0.5 mM. Two milliliters per liter of 0.002 mM ferrous sulfate was added twice weekly to supply iron. The complete solution was adjusted with dilute sulfuric acid to pH 5.0. No attempt was made to maintain the main nutrient levels.

Pots were continuously aerated by bubbling air through the solutions during plant growth. Plants were grown for one week.

Extraction and assay of NR was by a modification of a previously published method (Hageman and Flesher, 1960). Approximately 0.7 g of fresh, green leaf tissue was excised per pot and suspended in an extraction medium consisting of 33 mM Tris buffer, 3.3. mM cysteine-HCl and 100 mM Na_4EDTA, adjusted to pH 7.2 and stored near 0 C at all times. The extract was filtered through glass wool and the volume was brought to 15 ml with extracting medium. It was centrifuged at 20,000g for 15 minutes at 0-4 C. The supernatant was stored at or below 0 C until assay.

The assay was preformed in two tubes, an assay tube and an enzyme blank. The assay tube contained 1 ml of 0.05 M phosphate buffer (pH 7.0), 0.2 ml of 0.1 M KNO_3, and 0.5 ml of 1.3 mM NADH solution. In the enzyme blank, the NADH solution was replaced by 0.5 ml of the phosphate buffer. One ml of enzyme extract was added to each tube. The reaction was stopped by addition of 1 ml of 1% (w/v) sulfanilic acid in 3 N HCl and 1 ml of 0.02% (w/v) N-1-naphtylethylene-diamine HCl after 20 minutes. After 15 minutes the tubes were centrifuged for ten minutes and the absorbance at 540 nm was read on a Beckman DB Spectrophotometer.

It was subsequently found that the Hageman-Flesher technique used only 0.1 mM EDTA in the extraction medium. A brief experiment was conducted to consider the effect of this change on assay results. Plants were grown in vermiculite as described but were not transplanted to hydroponic solution. Instead they were maintained in vermiculite watered with nitrate-containing nutrient solution. After one week the plants were harvested and extracted in media as described except that EDTA was used

at three levels; 100 mM, 10 mM and 0.1 mM. The assay proceeded as described.

Carbohydrate was measured by the phenol-sulfuric method described by Dubois et al. (1956). Fifty ul of extract was placed in a test tube, 1.0 ml of 5% (w/v) phenol and 5 ml of 95% H_2SO_4 were added rapidly and the solution was mixed well. After cooling the solution, the absorbance at 480 nm was read.

Soluble protein was assayed on the 15% TCA precipitable fraction from 50 ul of extract using the Lowry method according to Miller (1959).

Field Studies

Soil samples were taken at fifteen locations in Kansas during the third week in May 1978. These locations contained the Kansas wheat variety performance tests. Four were taken at the 0 to 15-cm and the 15 to 46-cm depths and pooled from each replication at each location. The samples were analysed by the Kansas State University Soil Testing Laboratory.

Exchangable potassium was determined by extracting 5 g of dry soil with 25 ml of 1.0 N ammonium acetate, shaking the extract for 10 min., then filtering it through Whatman #2 paper. The potassium was then determined on a Perkin-Elmer 460 atomic absorbtion spectrophotometer.

Extractable phosphorus was determined by shaking 1 g of dry soil with 10 ml of a solution containing 30 mM ammonium flouride in 25 mM HCl for 40 sec and filtering it through Whatman #2 paper. Five ml of filtrate was removed and 5 drops of a second solution were added. This second solution was made by dissolving 100 g of ammonium molybdate in 850 ml of

water and filtering. To this a solution made by mixing 1.7 liters of conc. HCl and 160 ml of water was added. Finally, 110 g of boric acid was added to complete the mixture. After thorough mixing of the filtrate and the second solution, 5 drops of a third solution were added. This solution was made by mixing 5.0 g of sodium sulfite, 2.5 g of N-1-amino-2-napthol-4-sulfonic acid and 146.25 g of sodium bisulfite and grinding the mixture to a fine powder. Eight grams of this powder were dissolved in 50 ml of water before use. After mixing, the assay tubes stood for 15 min before reading the absorbance at 660 nm on a Coleman Spectronic 20.

Soil ammonium and nitrate were extracted by mixing 2 g of soil in 2 N KCl for one hour and filtering the mixture through Whatman #2 paper. Ammonium and nitrate were analysed on a Technicon Autoanalyser II.

Organic matter was determined by mixing 1 g of dry soil with 10 ml of 1 N potassium dichromate and 20 ml of conc. H_2SO_4 and allowing the solutions to stand for 30 min. One hundred ml of distilled water were added and the solution was filtered after cooling. The absorbance at 620 nm was read on the Coleman Spectronic 20.

The grain was harvested and weighed by the trial collaborators. Grain samples were taken and protein was analysed on the Technicon Infrared Reflectance Analyser in the Grain Science Department at Kansas State University. The results were corrected to 14% moisture.

Data Analysis

All the data was analysed using the SAS techniques described by Barr et al. (1976). Hydroponic data were analysed with analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) procedures. A soil mineral content-grain protein model was constructed using a general linear model (GLM) procedure. The program used a least-squares method to fit the model line to the data. Using an F-test, the less important factors in the model were sequentially removed until a suitable model was developed. Various interactions between soil nutrients and wheat varieties were tried in the model and eliminated in the same manner.

It will be noted that the final coefficients for location and variety are zero. In these cases the computer program set these parameters equal to zero and calculated the comparative effects of the other parameters of the same type. In these cases the absolute effect has been absorbed into the overall mean. In the protein yield model the P 46-cm by variety and NO_3 46-cm by variety effects are not compared to any value. In this case the parameter for comparision is not in the model by itself. The computer set the parameter to zero and computed the actual slopes of the response lines.

RESULTS

Hydroponic Studies

The results of the nitrate reductase assay are shown in Table 1. Solution nitrate and potassium levels did not show any statistically significant effect.

TABLE 1

Nitrate reductase activity.

potassium	nitrate	Phosphorus mM 0.005	0.01	0.05	0.1	0.5
mM		-----umoles NO_2^- min^{-1} g-fr.wt.$^{-1}$----------				
0.05	5.0	.39	.47	.46	.46	.28
	10.0	.51	.44	.48	.42	.41
	15.0	.46	.34	.45	.49	.45
0.5	5.0	.51	.44	.49	.52	.58
	10.0	.48	.43	.33	.51	.51
	15.0	.39	.36	.55	.64	.38
5.0	5.0	.38	.51	.51	.56	.36
	10.0	.50	.45	.51	.73	.45
	15.0	.38	.54	.47	.50	.64

Solution phosphorus did show a significant effect.

The highest NR activity was found in plants treated with 0.05 mM and 0.1 mM phosphate.

All other treatments had significantly lower enzyme activity and could not be distinguished from each other. (Table 2). There were no significant interactions between any of the nutrients to produce a higher NR activity.

The carbohydrate assays revealed no significant response of soluble carbohydrate to nutrient level (Table 3). There were no significant interactions between any of the nutrients to produce higher carbohydrate concentrations.

The response of protein to solution nutrients is shown in Table 4. Again, nitrate levels had no significant effect on soluble leaf protein content. Low phosphorus (0.01 mM) produced the highest protein concentration (Table 5). Low K (0.05 mM) produced the highest protein while moderate K (0.5 mM) produced the lowest protein concentration. Highest K (5.0 mM) produced an intermediate protein concentration and could not be distinguished from either of the other treatments. (Table 6). The only interaction which showed a significant effect on protein concentration was between low K (0.05 mM) and lowest P (0.05 mM). This is shown in Table 7.

Multivariate analysis of the data indicated that soluble carbohydrate and NR activity were negatively correlated (Table 8) but all other correlations were not significant.

TABLE 2

The overall means of NR activity at five levels of phosphorus nutrition.

PHOSPHATE	MEAN NR ACTIVITY	GROUPING
mM	umoles NO_2^- min^{-1} g-fr.-wt.$^{-1}$	alpha 0.05
0.005	0.44	A
0.01	0.44	A
0.05	0.46	A B
0.1	0.53	B
0.5	0.45	A

TABLE 3

Soluble Carbohydrate

		Phosphorus mM				
potassium	nitrate	0.005	0.01	0.05	0.1	0.5
mM		mg g-fr.-wt.$^{-1}$				
0.05	5.0	39.0	40.0	39.3	53.7	53.7
	10.0	41.7	40.0	23.0	19.7	26.0
	15.0	58.7	37.0	26.7	24.7	25.7
0.5	5.0	17.3	46.6	35.0	24.7	24.3
	10.0	39.3	25.3	38.0	17.7	14.0
	15.0	40.0	19.7	23.3	34.3	18.0
5.0	5.0	45.0	22.3	31.7	27.3	28.7
	10.0	46.7	20.0	21.3	111.0	16.0
	15.0	27.3	53.0	33.3	24.0	34.7

TABLE 4

Soluble leaf protein.

potassium	nitrate	Phosphorus mM 0.005	0.01	0.05	0.1	0.5
mM		mg g-fr.-wt.$^{-1}$				
	5.0	81.3	45.3	47.0	27.7	27.0
0.05	10.0	39.3	33.0	16.7	20.3	39.3
	15.0	53.0	45.3	29.7	23.0	26.0
	5.0	78.0	51.0	35.3	31.0	22.7
0.5	10.0	21.3	26.3	19.3	34.0	46.7
	15.0	19.0	16.0	21.3	39.3	30.3
	5.0	80.3	24.0	32.3	37.0	20.7
5.0	10.0	24.0	49.7	33.3	40.7	36.3
	15.0	64.7	14.3	30.3	36.0	65.0

TABLE 5

The overall mean protein concentration at five levels of phosphorus nutrition.

Phosphate	Protein Content	Grouping
mM	mg g-fr.-wt.$^{-1}$	alpha 0.05
0.005	51.2	A
0.01	33.9	B
0.05	29.5	B
0.1	32.1	B
0.5	34.9	B

TABLE 6

The overall mean protein content at three levels of potassium nutrition.

Potassium	Protein Content	Grouping
mM	mg g-fr.-wt.$^{-1}$	alpha 0.05
0.05	42.7	A
0.5	32.0	B
5.0	34.2	A B

TABLE 7

The interaction of phosphorus and potassium on mean protein content.

Phosphorus	Potassium	Protein Content	Grouping
mM	mM	mg g-fr.-wt.$^{-1}$	alpha 0.05
0.005	0.05	79.9	A
0.01	0.05	40.1	B
0.05	0.05	38.2	B
0.1	0.05	31.9	B
0.5	0.05	23.4	B
0.005	0.5	28.2	B
0.01	0.5	36.3	B
0.05	0.5	23.1	B
0.1	0.5	31.7	B
0.5	0.5	40.8	B
0.005	5.0	45.5	B
0.01	5.0	25.2	B
0.05	5.0	27.1	B
0.1	5.0	32.8	B
0.1	5.0	40.4	B

TABLE 8

Partial correlation coefficients between NR activity, protein content, and carbohydrate content over all treatment.

	Carbohydrate	Protein	NRactivity
Carbohydrate	1.000	-0.013	-0.264*
Protein		1.000	-0.014
NRactivity			1.000

* significant at the 0.05 level

Results of the brief experiment determining the effect of EDTA on nitrate reductase activity are shown in Table 9. While a definite effect was noted, it was decided to proceed using the data collected. These data indicate that the extraction procedure is the largest variable in the experimental procedure.

Field Studies

Soil Data

The mean soil nutrient results for each location are shown in Tables 10 and 11. Also shown are the mean grain protein percentages over all varieties for each location. These mean values represent the data which was used to construct the two protein models.

A Soil-Grain Protein Model

Through addition and subtraction of various parameters the following general linear model (Equation 1) was found to best relate soil fertility and grain protein percentage.

$$P_{ijk} = u + L_i + V_j + b_1\ (\text{K 15-cm}) + e_1\ (\text{K 15-cm}) + b_2\ (\text{K 46-cm}) + b_3(\text{NH}_4\ \text{46-cm}) + b_4(\text{NO}_3\ \text{46-cm}) + b_5(\text{OM 15-cm}) + b_6(\text{OM 46-cm}).$$

(Equation 1)

P_{ijk} is the predicted protein percentage, u the overall mean, L_i is the location effect, and V_j is the variety effect. The coefficients, b_1 through b_6, describe the magnitude of the effect of a one-unit increase in the soil parameter. The coefficient, e_1, describes the interaction between a specified variety and an increase in the soil parameter.

TABLE 9

The effect of EDTA concentration on NR extraction and activity in three extraction trials.

EDTA	mean nitrate reductase activity in three assays			mean
mM	---------umoles NO_2^- min^{-1} g-fr.-wt.$^{-1}$----------			
0.1	1.15	0.56	0.81	0.84
10.0	0.56	0.41	0.74	0.57
100.0	0.23	0.43	0.35	0.34

TABLE 10

Mean soil nutrient levels in the 0 to 15-cm profile.

Location	Phosphorus	Potassium	NH_4-N	NO_3-N	Org. Matter	Mean Grain Protein
	----------ppm----------				%	%
Ft. Hays	23.6	700	4.7	9.2	1.9	12.7
Colby(irr.)	14.5	715	4.9	13.8	1.3	11.9
Colby	20.3	693	3.3	10.9	1.3	10.7
Tribune	20.1	570	3.2	6.7	1.0	10.9
Tribune(irr.)	27.6	654	4.5	10.0	1.7	12.7
Garden City	35.6	673	2.8	5.7	1.1	10.8
Garden City (irr.)	9.6	592	2.9	9.6	1.7	12.4
Mineola	10.3	715	5.8	11.7	1.8	12.7
Hutchinson	16.4	261	3.7	5.3	1.6	10.5
Hesston	5.8	342	6.8	9.0	1.9	13.3
Parsons	3.3	103	6.5	36.1	2.8	13.9
Ottawa	7.4	185	6.1	26.5	2.5	14.7
Ashland	13.1	226	6.1	8.0	1.5	11.1
Belleville	33.3	400	5.3	7.1	2.1	12.5

TABLE 11

Mean soil nutrient levels in the 15 to 46-cm profile.

Location	Phosphorus	Potassium	NH_4-N	NO_3-N	Org. Matter	Mean Grain Protein
	ppm				%	%
Ft. Hays	6.4	527	4.7	7.9	1.2	12.7
Colby (irr.)	13.7	693	6.5	12.8	1.2	11.9
Colby	15.9	652	3.8	9.1	1.1	10.7
Tribune	7.5	536	2.9	5.7	0.9	10.9
Tribune (irr.)	14.3	555	4.1	7.9	1.6	12.7
Garden City	14.3	493	2.8	5.1	0.9	10.8
Garden City (irr.)	9.8	568	3.4	12.1	1.5	12.4
Mineola	41.5	443	4.7	7.5	1.3	12.7
Hutchinson	20.3	243	3.7	4.4	1.6	10.5
Hesston	32.5	326	3.8	11.6	1.3	13.3
Parsons	17.6	84	4.7	14.2	1.6	13.9
Ottawa	42.6	180	5.5	15.1	1.8	14.7
Ashland	6.9	172	4.1	6.8	1.2	11.1
Belleville	12.5	332	15.8	6.0	1.6	12.5

The remaining variables represent measured soil nutrient levels in ppm. Table 12 gives the values of the main effect and interaction coefficients. These values can be used in the following example to predict the protein percentage of Lancota wheat grain grown at Ft. Hays:

$$P_{ijk} = 8.94 - 1.27 + 1.94 + 0.005\,(700) - 0.001\,(700) + 0.001(527)$$
$$-0.007(4.7) + 0.006(7.9) - .301\,(1.9) + 876(1.2)$$
$$= 13.4\%$$

(Equation 2)

This compares with the observed Lancota mean protein percentage at Ft. Hays of 13.6. The certainty of the choice of parameters can be illustrated in Table 13. The column labled PR F gives the chance that one would be correct in eliminating that factor from the model.

Small values indicate high importance. A more precise comparison can be gained from the F-value column by consultating a F-table with the appropriate number of degrees of freedom.

A model of protein yield was constructed in the same manner; the general equation is shown below (Equation 3):

$$Y_{ijk} = u + L_i + V_j + b_1(\text{K 15-cm}) + b_2(\text{OM 46-cm}) + e_1(\text{P 46-cm})$$
$$+ e_2(NO_3\ \text{46-cm})$$

(Equation 3)

Y_{ijk} is the predicted protein yield in pounds per acre. The overall mean is u; L_i is the location effect; and V_j is the variety effect. The coefficients have the same means as described above. The main effect

TABLE 12

Parameters and coefficients for the general linear model of protein percentage

Parameter	Coefficient	Parameter	Coefficient	
Overall Mean				
Location			Variety	K 15 cm by Variety
Ft. Hays	-1.266	Bennett	0.714	0.000
Colby (irr.)	-2.550	Buckskin	0.116	0.000
Colby (dry)	-3.513	Centurk	0.058	0.000
Tribune (dry)	-2.456	Centurk 78	-0.153	0.001
Tribune (irr.)	-1.354	Cheney	0.782	-0.001
Garden City (dry)	-2.933	Eagle	1.237	-0.001
Garden City (irr.)	-1.564	Lancota	1.946	-0.001
Mineola	-1.309	Larned	0.472	-0.001
Hutchinson	-1.464	Lindon	-0.004	0.000
Hesston	1.100	Newton	0.212	0.000
Parsons	2.962	Osage	1.136	-0.001
Ottawa	3.034	Payne	0.704	0.000
Ashland	-0.363	Sage	1.196	-0.001
Belleville	0.000	Scout	0.618	-0.001
		Tam W-101	0.712	0.000
K 15 cm	0.005	Vona	0.000	0.000
K 46 cm	0.001			
NH_4 46 cm	-0.007			
NO_3 46 cm	0.016			
Org. matter 15 cm	-0.301			
Org. matter 46 cm	0.876			

Table 13

Analysis of varience table for the protein percentage general linear model.

Source	degree of freedom	sum of squares	F-value	PR F
Location	13	278.21	61.01	0.0001
Variety	15	45.17	8.58	0.0001
K 15 cm	1	14.69	41.88	0.0001
K 46 cm	1	1.40	4.00	0.0459
NH_4 46 cm	1	1.01	2.87	0.0905
NO_3 46 cm	1	0.72	2.06	0.1515
K 15 cm by variety	15	18.41	3.50	0.0001
Org. matter 15 cm	1	1.00	2.84	0.0920
Org. matter 46 cm	1	6.77	19.30	0.0001

and interaction coefficients are shown in Table 14. The analysis of variance table (Table 15) gives the values for the certainty of the factor's importance.

DISCUSSION

Hydroponic Studies

Leaf nitrate reductase did not seem to be strongly affected by nutrient availability. The lack of a significant nitrate effect is particularly surprising. Though a trend suggested that higher nitrate might cause higher enzyme levels, the trend did not correspond to the change in nitrate availability. Several factors might be responsible. The lowest nitrate level supplied, 5 mM, might have been adequate for the plant's needs and induced maximum enzyme activity. Also, it may be due to limited nitrate uptake which is matched to the plant's reduction capacity (Butz and Jackson, 1977; Chantarotwong et al., 1976). Nitrate may induce high enzyme concentrations which are then subject to *in vivo* regulation (Chantarotwong et al., 1976). The *in vivo* assay used in these studies would not show this type of regulation.

Though phosphate is known to enhance enzyme activity *in vivo* (Kinsky and McElroy, 1958; Ferrari and Varner, 1970), these effects cannot explain the observed phosphate as phosphate was available in the assay medium. At low phosphate availability, the lack of high energy phosphate may limit NR synthesis or nitrate uptake (Butz and Jackson, 1977). At adequate phosphate levels the enzyme is present at higher levels. Very high phosphate levels may lead to accumulation of free amino acids or secondary products which act to repress NR synthesis

TABLE 14

Parameters and coefficients for the general linear model of protein yield.

Parameter	Coefficient	Parameter	Coefficient		
			Variety	P 46 cm by Variety	NO_3 46 cm by Variety
Overall mean					
Location	219.50	Bennett	25.00	-0.97	-2.43
Ft. Hays	- 96.92	Buckskin	- 0.36	-0.02	-3.86
Colby (irr.)	29.58	Centurk	-12.03	-0.07	-2.74
Colby (dry)	- 36.38	Centurk 78	-17.49	-0.28	-0.84
Tribune (dry)	- 78.93	Cheney	-28.53	-0.18	-0.85
Tribune (irr.	149.07	Eagle	-32.83	-0.06	-0.14
Garden City (dry)	- 67.42	Lancota	4.50	-0.73	-1.99
Garden City (irr.)	74.47	Larned	- 9.57	-0.58	-1.51
Mineola	-126.89	Lindon	- 6.24	0.26	-3.47
Hutchinson	- 70.59	Newton	-29.67	1.08	0.91
Hesston	129.61	Osage	-41.90	-0.44	0.27
Parsons	- 10.05	Payne	-10.36	-0.09	2.55
Ottawa	- 76.69	Sage	-38.86	-0.61	3.08
Ashland	10.91	Scout	-22.81	-0.66	-0.88
Belleville	0.00	Tam W-101	0.58	-0.57	-0.44
		Vona	0.00	-0.31	-2.67
K 15 cm	0.10				
Org. matter 46 cm	45.27				

TABLE 15

Analysis of variance table for the protein yield general linear model.

Source	Degrees of Freedom	Sum of Squares	F-Value	PR F
Location	13	5901070	264.50	0.0001
Variety	15	48661	1.89	0.0210
K 15 cm	1	10509	6.12	0.0135
Org. matter 46 cm	1	23726	13.83	0.0002
P 46 cm by variety	16	37153	1.35	0.1584
NO_3 46 cm by variety	16	69033	2.51	0.0009

(Filner, 1966; Joy, 1969; Radin, 1977; Schrader and Hageman, 1967).

Potassium had no effect on NR activity. This observation matched that of Hsiao et al. (1970), who found no direct effect of potassium on NR activity. This is contrasted with the findings of Frost et al. (1978), who showed a three-fold increase of NR activity in K-treated plants over K-deprived plants. This contradiction might be explained as the effect of potassium on nitrate uptake rather than on the enzyme. Complete potassium deprivation may restrict nitrate uptake and prevent NR induction (Shaner and Boyer, 1976). Minimal levels of potassium may allow nitrate uptake and reduction at rates not very different from rates at potassium levels of luxury comsumption (Hsiao et al., 1970).

Soluble carbohydrate showed no response to nutrients in this experiment. This is in contrast to earlier experiments (Mosolov and Volleidt, 1962). One explanation for this observation may be that young, rapidly growing seedlings do not accumulate significant amounts of low molecular weight sugars. Rapid export or incorporation of photosynthate into structures may mask any regulation of carbohydrate flow by these nutrients.

The protein response of leaves with varying nutrition is difficult to explain. Nitrate, which should affect protein concentration, had no effect. Both low potassium (0.05 mM) and high potassium (5.0 mM) led to high protein as did low phosphorus (0.005 mM). High potassium may act by increasing nitrate uptake and transport (Frost et al., 1978). The effect of low potassium matches earlier reports (Hsiao et al., 1970) but no mechanistic explanation is apparent. The high protein effect of low phosphorus was earlier shown by Mosolov and Volleidt (1962) and Harper

and Paulsen (1969). The former attributed the effect to increased availability of carbohydrate for amino acid synthesis. Low phosphorus and low potassium apparently interacted to produce high protein. This interaction was also seen in the data of Mosolov and Volleidt (1962). It seems unlikely that the two conditions act through a single mechanism. It is more likely that the two effects occur at different points in the metabolic pathways to protein and act additively.

The partial correlation coefficients indicate a negative relationship between soluble carbohydrate and NR activity. Low carbohydrate concentrations might limit mineral uptake and general protein synthesis. High NR activity may lead to increased amino acid synthesis, depleting the soluble carbohydrates in the cell.

Soil-Grain Protein Model

The model for protein percentage and protein yield shows that soil nutrient content influenced protein production. Soil nitrate had a moderate effect on protein percentage. This may be attributed to a general stimulation of nitrogen metabolism. The fact that nitrate was no more than moderately important probably indicated that nitrate was sufficient in many soils and other factors limited protein production. The negative effect of ammonium is less clear. This is probably coincidental but it may be related to competition with potassium for uptake sites. In nitrate-sufficient soils, potassium may be preferentially taken up by the root. Many of the soils in this study had reasonable levels of nitrate and high levels of potassium.

The effect of potassium in stimulating protein production may be related to stimulation of nitrate uptake as described by Frost et al. (1978). Acting as a counterion, potassium contributes in a small but very significant way to the control of protein synthesis. This effect was not a function of variety as no strong positive relationship between high protein wheats and potassium was noted. The lower level of importance for potassium in the 15 to 46-cm portion of the profile may be related to the development of the crop. By the time the roots are exploiting the lower portion of the profile the plant may be nearly satiated with potassium. Further additions of potassium are stored in the vacuole and have no effect on nitrate transport.

The effect of organic matter would not seem to be through any direct nutritional response. However, release of available nitrogen by microbial action could result in major additions of soil nitrogen as well as mobilize other soil nutrients (Brady, 1974). This latter factor may be very important on the low potassium, low phosphorus soils found in the study. It should be noted that Ottawa and Parsons, which had the lowest potassium levels, had the highest soil nitrate, soil organic matter, and grain protein concentrations.

The lack of any phosphorus effect on protein percentage may be due more to lack of soil availability (Brady, 1974) and lack of soil variability than to a lack of a plant response. Mosolov and Volleidt (1962) showed a distinct effect of high phosphorus on grain protein concentration in a greenhouse study. These phosphorus availability problems may explain the mixed results (Leikem et al., 1978; Murphy et al., 1977)

obtained by previous investigators. Addition of high levels of phosphorus fertilizers do not always result in high levels of available soil phosphorus (Brady, 1974). Interactions with soil microbes may tightly regulate phosphorus availability (Brady, 1974).

The protein yield model should include all the factors which affect protein percentage plus any factors which influence yield. However, this is not the case with this protein model. The nitrate content of the 15 to 46-cm portion of the profile dropped out of the model. This may relate to the fact that nitrate at the late date of sampling no longer has an effect on yield. Yield effects of nitrogen are usually noted with early applications of nitrogen. Late application of nitrogen may stimulate protein concentration but have no effect or a negative effect on grain yield. This may be reflected in the fact that nitrate variety interactions were predominately negative. The degree of certainty for this observation was relatively high. Generally varieties that had highest yield of protein had the most negative response to nitrate, while varieties that had lowest protein yield responded most positively to nitrate.

Potassium in the 15 to 46-cm portion of the profile also dropped out of the model. This probably was due to satiation of the yield response with potassium. As the effect on protein percentage was not extremely strong, this parameter might not be carried through the model. The potassium in the upper 15-cm portion of the profile was still very important and probably the effect as a counterion was carried through to protein yield in this case.

While phosphorus was not an important factor in the yield model, its interaction with the varieties was marginally important. The mechanism of this interaction is not clear and the possibilities are complex (Sorenson, 1971). Again, varieties that had the highest protein yields had the most negative response to increasing phosphorus levels.

The effect of organic matter in the upper 15-cm of the profile on yield was negligible. This may reflect the fact that the effect on protein percentage was marginally important. The organic matter in the 15 to 46-cm portion of the profile was very important. This may be due to the fact that little of the nitrogen in the organic matter is released during a growing season and, correspondingly, the organic matter levels do not change much in a season. In this event the organic matter measurement can reflect the early season effects. Nitrogen from organic matter decomposition is available early in the season and can positively affect yields.

Throughout this discussion, the soil parameters have been considered in the absence of climatic factors. This is a severe flaw when considering as indeterminant a thing as yield or protein percentage. The model, at this stage, is incapable of distinguishing between a soil parameter and the climatic conditions associated with that soil condition. An example of this effect might be the realtionship between soil potassium and precipitation:evaporation ratio. A number of the soils which had high potassium levels were in areas with high precipitation:evaporation ratios. One can legitimately ask if high soil potassium increased protein production or if drought stress limited

starch synthesis, which resulted in a high protein percentage. Varieties from three irrigated sites had both higher grain protein percentages and higher protein yields than varieties from non-irrigated sites. The difference in soil characteristics is not sufficient to explain the differences. As a result of these sorts of effects, the location factor was a catch-all for factors which were not otherwise included in the model. Certain errors of this type might be eliminated by collecting data over a number of years and by including climatic information from each site.

A further flaw in the model is that it was fitted to the data. A cause-and-effect model would predict that increasing application of a fertilizer would increase grain protein concentration. A coincidence model, like this one, says that soils which had high levels of the particular nutrient also produced high grain protein levels.

A final flaw in using this sort of linear model is that response curves are not detected. A nutrient at very high levels may lead to decreasing response while at low levels it may lead to increasing response. The result is that the parameter is discarded from the model as not being an important predictor of the response. A different sort of model might not have this limitation.

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EFFECT OF NITROGEN, PHOSPHORUS AND POTASSIUM ON WHEAT (*TRITICUM AESTIVUM* L.) GRAIN PROTEIN

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B.S., University of Minnesota, 1975

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Agronomy

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1979

Wheat grain yields and grain protein concentrations are usually inversely related. Recent claims state that high protein wheats which exhibit normal yield characteristics can be bred. Studies on these wheats involved very high fertilization levels. This study was done to determine the effect of plant nutrient levels on grain protein. The experiment had two parts. A hydroponic study involving the wheat variety "Plainsman V" grown under three nitrate levels, five phosphate levels, three potassium levels and all their combinations tested the effect of the nutrients on *in vitro* nitrate reductase activity, soluble leaf carbohydrate concentration and soluble leaf protein. Nitrate reductase was affected only by phosphate level; 0.05 mM and 0.1 mM concentrations gave the highest activity. High phosphate (0.5 mM) and low phosphate (0.005 mM and 0.01 mM) concentrations decreased enzyme activity. Soluble leaf carbohydrate was not affected by nitrate, phosphorus or potassium level in the hydroponic medium. Soluble leaf protein was increased by low phosphorus (0.005 mM), low potassium (0.05 mM) and high potassium (5.0 mM). Potassium counterion effects apparently stimulated nitrogen metabolism. An interaction between low phosphorus (0.005 mM) and low potassium (0.05 mM) acted to increase leaf protein content. No mechanism can clearly explain this result. An experiment involving sixteen hard red winter wheat varieties grown in fourteen locations in Kansas was also conducted. This experiment investigated the effect of soil nutrient levels on grain protein concentration and protein yield. A computer procedure established a general linear model which fitted either protein percentage or protein yield to soil nutrient data. Discriminatory procedures eliminated soil nutrients which did not have significant effects in predicting the protein response. On the basis of this analysis a tentative model for predicting protein percentage and

protein yield (percentage x grain yield) was constructed. The model showed that protein percentage can be increased by 0.001% for each ppm increase in potassium. One ppm of nitrate could increase protein percentage by 0.016%. Organic matter, probably through nitrification, increased protein percentage by 0.876% for each percent increase of organic matter. Differences in varietal protein production and varietal interaction with soil nutrient levels were observed. Protein yield was somewhat less responsive to soil nutrient level. That may have been due to the many yield factors set early in the season before soil samples were taken at or after anthesis. The lack of consideration of climatic date introduced potentially large errors in consideration of location effects.