Nitrate Reductase of Wheat — Its Relation to Nitrogen Fertilization[1]

H. H. HERNANDEZ[2], D. E. WALSH[3], and A. BAUER[3], North Dakota
State University, Fargo 58102

ABSTRACT

Nitrate reductase, a rate-limiting enzyme in wheat protein synthesis, was highly influenced by wheat varieties and nitrogen fertilization. Seasonal patterns for three wheat varieties showed that nitrate reductase activities of hard red spring wheat leaf tissues were low in late May (2–3-leaf stage), reached a maximum in early June (boot stage), and diminished rapidly in late June and early July as the wheat began to set seed. Waldron, a tall hard red spring wheat variety, showed lower average reductase activities throughout the growing season than World Seeds 1809 and Bounty 208, two semidwarf hard red spring wheat varieties. Nitrate reductase activity, determined at the boot stage of wheat maturity, showed highly significant positive correlations (1% level of confidence) between wheat grain yield and grain protein yield. Nitrate reductase activity as well as wheat protein yield increased with application of nitrogen fertilizer. Significant protein yield increases were noted with 36 and 64 lb. of nitrogen per acre. However, fertilization with 140 lb. of nitrogen failed to show a significant increase in protein over the 70 lb. per acre of nitrogen fertilizer treatment.

The shortage of protein for the human diet is well known to those familiar with world food production problems. Since cereal grains provide nearly one-half the world's food calories and one-third of the protein, it is obvious that any increase in protein quality or quantity in cereals would greatly improve human nutrition throughout the world.

Soil scientists, plant breeders, and cereal chemists have spent years of research to improve wheat crops. However, many questions regarding the quality of wheat proteins remain unanswered. The major factors influencing wheat protein quality are yet to be defined completely. Little is known concerning the mechanism by which wheat protein content can be increased.

According to Schiller et al. (1), climate, soil fertility, and genotype are the main factors which influence both protein quality and quantity in wheat. Furthermore, these three factors appear to be interdependent. One limiting factor in the synthesis of protein is the ability of the wheat plant to utilize available nitrogen in the soil. Since soil nitrate is the major form of available nitrogen, the wheat plant must possess an active mechanism by which nitrate is absorbed and reduced to a form which can be incorporated into amino acids for protein synthesis. In green plants, the enzyme nitrate reductase appears to be the rate-limiting enzyme in the series of reactions whereby nitrogen is utilized for protein synthesis. Nitrate reductase is known to be influenced by available soil nitrogen (2), the amount of light (3), various other environmental conditions (4), and the genetic composition of wheat (5).

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[2]Present address: Direccion de Extension Agricola, Departmento de Suelos, Balderos No. 94, Mexico 1, D. F.

[3]Respectively: Associate Professor of Cereal Chemistry and Technology and Professor of Soils.

The purpose of the present research was to investigate the influence of nitrogen fertilization on wheat nitrate reductase activity, and to measure the effects of variety and nitrogen fertilizer treatment on the yield and protein content of wheat.

MATERIALS AND METHODS

Plant Material

Three wheat varieties of diverse genetic composition were included in the study: Waldron, a conventional-height, hard red spring wheat variety; and Bounty 208 and World Seeds (W.S.) 1809, semidwarf wheats with lower protein contents. Seeding rates were the same for all wheat varieties with seeds spaced 1 in. apart in rows 7 in. apart. The wheat was planted April 29 in plots measuring 24 X 7 ft. Ten-gram samples of leaf tissue samples were harvested from one row in each of four replicated plots and analyzed for nitrate reductase activity at weekly intervals from the beginning (2-leaf stage) of the growing season until the wheat plants began to mature and set seed.

Fertilizer Treatments

Fertilizer was applied May 4 as ammonium nitrate by broadcast at the rates of 0, 35, 70, and 140 lb. nitrogen (N) per acre. Analysis of soil samples from the field plots showed that they contained 35, 64, 101, and 136 lb. N per acre, respectively, as NO_3 in the upper foot of soil on June 8. To assure adequate water for plant growth throughout the growing season, 2 in. of water was added by sprinkler irrigation on July 9 to give a total of 10 in. of water (rain plus irrigation) for the season.

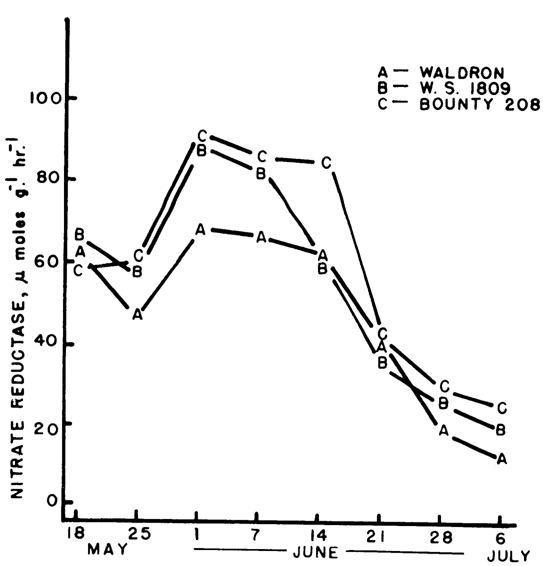

Fig. 1. Effect of wheat maturity on the nitrate reductase activity in wheat leaf tissues. Wheat maturity stages were as follows: 2 leaf, May 18; 3 leaf, May 25; 4 leaf, June 1; 5–6 leaf, June 7; boot, June 14; milk, June 21 to June 28; soft dough, July 6.

Assay Procedure

Nitrate reductase activity of the freeze-dried leaf tissue was determined by the method of Hageman and Flesher (6) method as modified by Eilrich (7). Tests of fresh and freeze-dried tissue showed no significant enzyme loss due to freeze-drying. Nitrate reductase activities are reported as μmoles of nitrite produced per hr. per g. dry plant tissue. Grain protein analyses were performed according to AACC Approved Methods (8).

RESULTS AND DISCUSSION

Nitrate Reductase Response to Maturity and Fertilizer

The nitrate reductase activity pattern for each wheat variety throughout the growing period is shown in Fig. 1. The seasonal patterns were similar for the three varieties. As the wheat grew, the leaf nitrate reductase activity increased initially to a maximum (between May 25 and June 1). Throughout the remainder of the growing season, a steady decline in activity was observed. Finally, in late June, when the plants began to set seed, the activity was very low.

The influence of soil nitrogen on nitrate reductase activity of spring wheat leaf tissue is shown in Fig. 2. There was a direct, positive relation between increasing nitrate reductase with increased rates of fertilizer nitrogen. All three varieties showed a continuous increase in enzyme activity throughout the range of fertilizer nitrogen tested. Waldron, W.S. 1809, and Bounty 208 showed linear correlations of r = 0.998, r = 0.984, and r = 0.935, respectively, for soil nitrate versus nitrate

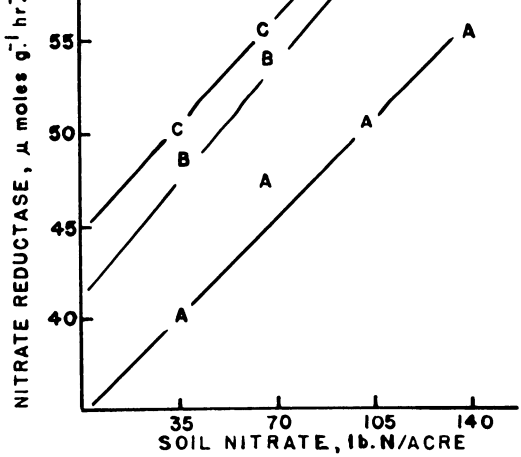

Fig. 2. Influence of soil nitrate content on average leaf tissue nitrate reductase activity of three wheat varieties. Data are expressed on a dry tissue basis.

reductase activity. Waldron was lower in activity than the two semidwarf varieties at all fertilizer levels. Bounty 208 and W.S. 1809 showed high nitrate reductase levels which ranged in activity from 46 to 65 μmoles g.$^{-1}$ hr.$^{-1}$ at 35 and 136 lb. of N (nitrate) per acre, respectively. Since none of the varieties leveled off in enzyme activity at the highest soil nitrogen levels tested, it appeared that greater nitrate reductase activity would result from increasing soil nitrogen beyond 136 lb. per acre. In addition, it was concluded that the semidwarf wheats possessed a more active mechanism to reduce nitrate than did the tall Waldron wheat variety.

Grain and Protein Yield

An inverse relationship between grain wheat yield and protein content has been observed (9). High-yielding wheat varieties often produce lower grain protein than their low-yielding counterparts. Apparently, the protein is diluted by large amounts of carbohydrates in the high-yielding wheat.

Figures 3 and 4 show the grain yield and protein yield responses of wheat to four levels of nitrogen fertilization. From Fig. 3 it was evident that Bounty 208 and W.S. 1809 yielded more bushels of wheat per acre than Waldron. Waldron increased linearly in yield with increasing nitrogen fertilization. However, even at the highest fertilizer level, the yield of Waldron was lower than the yields of W.S. 1809 and Bounty 208 at the lowest fertilizer levels tested. W.S. 1809 increased substantially in yield with nitrogen fertilization and had its highest yield (74.4 bu. per acre) with a fertilizer level of 101 lb. of nitrogen per acre. At 136 lb. of soil nitrogen per acre, the yield of W.S. 1809 dropped to 70.6 bu. per acre. The yield of Bounty 208 was not significantly changed with any of the nitrogen fertilizer levels tested.

Figure 4 shows the influence of soil nitrogen on the yield of protein per acre. For each variety an increase in protein yield occurred with each increase in the level of

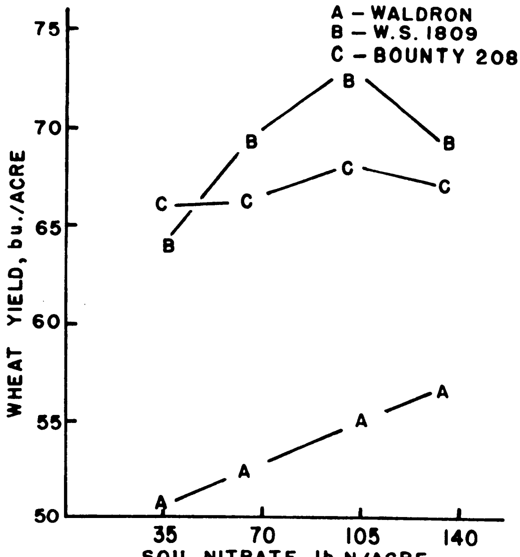

Fig. 3. Effect of soil nitrate content on wheat yield. Soil analyses are expressed as the total N in the top 12 in. of soil.

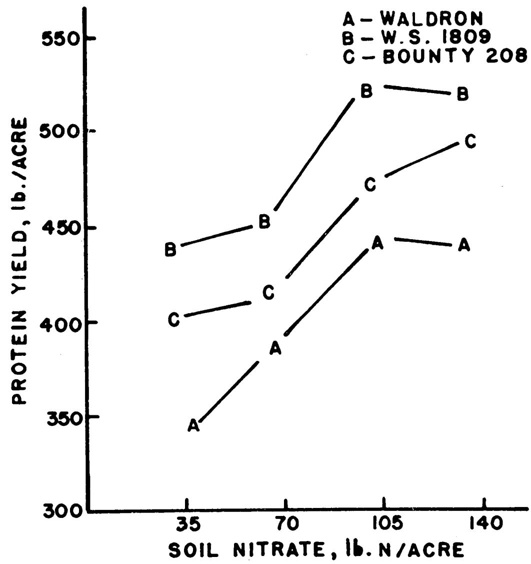

Fig. 4. Effect of soil nitrate content on wheat protein yield. Data are expressed on a dry tissue basis. Soil analyses are expressed as the N in the top 12 in. of soil.

soil nitrogen up to 101 lb. of nitrogen per acre. Waldron and W.S. 1809 showed a slight drop in protein yield when soil nitrate was increased above 101 lb. per acre. Waldron showed the lowest protein yields of the three varieties at all levels of soil nitrogen. These data confirmed the earlier conclusion reached from nitrate reductase data: the two semidwarf varieties had a more active mechanism to reduce nitrogen for protein synthesis than did Waldron.

Comparison of Fig. 1 with Fig. 4 reveals only a limited relation between nitrate reductase activity and protein yield. Waldron showed the lowest enzyme activities and the lowest protein yields per acre, whereas Bounty 208 and W.S. 1809 had higher nitrate reductase activities and protein yields.

Since the average nitrate reductase value appeared related to grain and protein yield, it was of interest to determine which stage of plant growth and nitrate reductase activity was most closelyassociated with grain and protein yields. Table I shows linear correlation coefficients for grain and protein yields versus average wheat leaf tissue nitrate reductase activities determined at weekly intervals throughout the growing season. The data showed that nitrate reductase was not closely related to grain yield and protein synthesis at the early stages of plant growth. However, during the late stages of growth, highly significant correlations were found.

Since correlation data show nitrate reductase activity is related to protein yield in the late stages of wheat plant development, it is apparent that wheat plants which maintain their nitrate reductase activity for a long period in plant development synthesize more protein than plants which have high activities for only a short period during the growing cycle. Consequently, nitrate reductase activity appears promising for prediction of wheat and protein yields if the test is

TABLE I. LINEAR CORRELATION COEFFICIENTS OF NITRATE REDUCTASE ACTIVITY VERSUS GRAIN AND PROTEIN YIELD

Sampling Date	Correlation between Nitrate Reductase Activity and: Grain Yield bu./acre	Protein Yield lb./acre
May 15	N.S.	N.S.
May 25	N.S.	N.S.
June 1	N.S.	N.S.
June 14	0.416**	0.524**
June 21	N.S.	0.311*
June 28	0.487**	0.583**
July 6	0.306	0.542**

TABLE II. ANALYSIS OF VARIANCE OF NITRATE REDUCTASE, WHEAT YIELD, PROTEIN YIELD, AND PROTEIN CONTENT DATA: EFFECT OF WHEAT VARIETIES AND FERTILIZER TREATMENTS

Source of Variation	Degree of Freedom	Mean Squares			
		Total Nitrate Reductase	Wheat yield	Protein yield	Protein content
Replications	3	3023.719	228.868**	4.214**	3.896**
Varieties (V)	2	34558.875**	1176.516**	8.888**	7.632**
Treatments (T)	3	36975.750**	69.111**	8.667*	11.930**
Var. V X T	6	449.581	12.360	0.255	1.545
Error	33	4672.712	12.115	0.410	0.8201

conducted during the late stages in plant development. If nitrate reductase is to be used for predicting protein yield the enzyme measurements should be done at or just after the boot stage in wheat development when the correlations between grain enzyme activity and protein yield are highly significant.

Table II shows an analysis of variance (AOV) for the average nitrate reductase activity, wheat yield, protein yield, and protein content data. The table confirms the conclusion that nitrate reductase, as well as wheat yield, protein yield, and protein content, was highly influenced by wheat varieties and fertilizer treatments. The AOV results agree closely with Fig. 2 which showed a highly significant response of enzyme activity to addition of nitrogen fertilizer. The highly significant effects of replications reflect variance caused by the environment in the field trials.

CONCLUSIONS

Nitrate reductase activity of wheat leaf tissue is highly influenced by nitrogen fertilization, wheat variety and the weather. Since the two semidwarf wheats were higher in nitrate reductase and yielded more bushels of wheat and more protein per acre than the conventional-height wheat, it was concluded that the semidwarf wheat possessed a more active mechanism to reduce nitrate for protein synthesis.

Wheat leaf tissue nitrate reductase assays appear promising as a method for predicting grain and protein yields. The highest correlations between nitrate reductase activity and grain and protein yields are achieved if the assay is performed at the boot stage of wheat development.

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