THE RELATION OF WHEAT NITRATE REDUCTASE AND SOIL NITRATE TO FLOUR QUALITY[1]

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

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ABSTRACT

Nitrate reductase activity of wheat-leaf tissues and soil nitrate content had a significant influence on flour protein content and bread-baking characteristics of hard red spring wheat. In replicated field trials at four levels of soil nitrate, the hard red spring wheat variety Waldron had the highest average flour protein content but the lowest average nitrate reductase activity of the three wheat varieties in the tests. Nitrate reductase was highly correlated with soil nitrate level, but there was a significant difference among wheat varieties. Waldron wheat in all trials was significantly lower in average nitrate reductase activity than was W.S. 1809 or Bounty 208 at the respective soil nitrate levels. Nitrate reductase activity was correlated with flour ash contents and dough-mixing score. However, large variations were noted among wheat varieties in the relation of nitrate reductase activity to flour and bread quality.

The protein of wheat has been recognized for many years as an important factor in determining bread-baking performance of wheat. Mangels and

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

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

Sanderson (1) collected samples from wheat crops from 1916 to 1924 and noted that bread quality was highly dependent on wheat protein content. Other workers have reported that wheat protein content was positively correlated with flour milling yield, color of bread, baking absorption, loaf volume, and dough-mixing characteristics (2–8).

Many factors, however, can influence the quantity and quality of wheat protein. Fertilization of the soil with nitrogen (N) increases the protein content of wheat (9) when more fertilizer nitrogen is applied than is needed for grain yield increase (10). The genetic background of wheat variety can have a marked influence on protein content and bread-baking characteristics of wheat (11).

In green plants, nitrate reductase appears to be a rate-limiting enzyme in a series of reactions whereby available soils nitrogen is used for protein synthesis. Recently, Hernandez *et al.* (12) demonstrated that nitrate reductase activity was

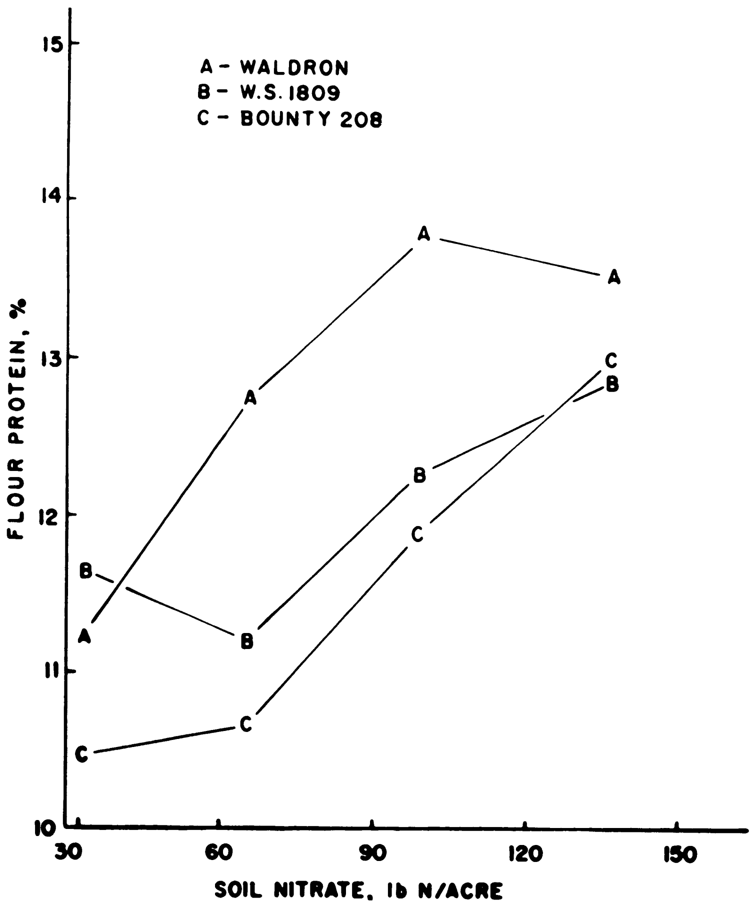

Fig. 1. Effect of soil nitrate level on wheat-flour protein content. Protein content is on a 14% flour moisture basis and soil data are expressed in lb N/acre, as nitrate in the top 12 in. of soil on June 8.

positively correlated with wheat protein content and the ability of wheat varieties to use soil nitrate for protein synthesis. The purpose of the present work was to investigate the relation of wheat-leaf tissue nitrate reductase and soil nitrate level to the milling and bread-baking characteristics of three hard red spring (HRS) wheat varieties.

MATERIALS AND METHODS

Plant Material

Three HRS varieties of diverse genetic composition were included in the study: Waldron, of conventional height, and Bounty 208 and World Seeds (W.S.) 1809, both semidwarfs of lower protein content than Waldron. The wheats were planted April 29, 1971, at the North Dakota Agricultural Experiment Station at Carrington, N. Dak., in rows 7 in. apart with seeds spaced about 1 in. apart. Each plot measured 24 × 7 ft. The main treatments of the split plot design were

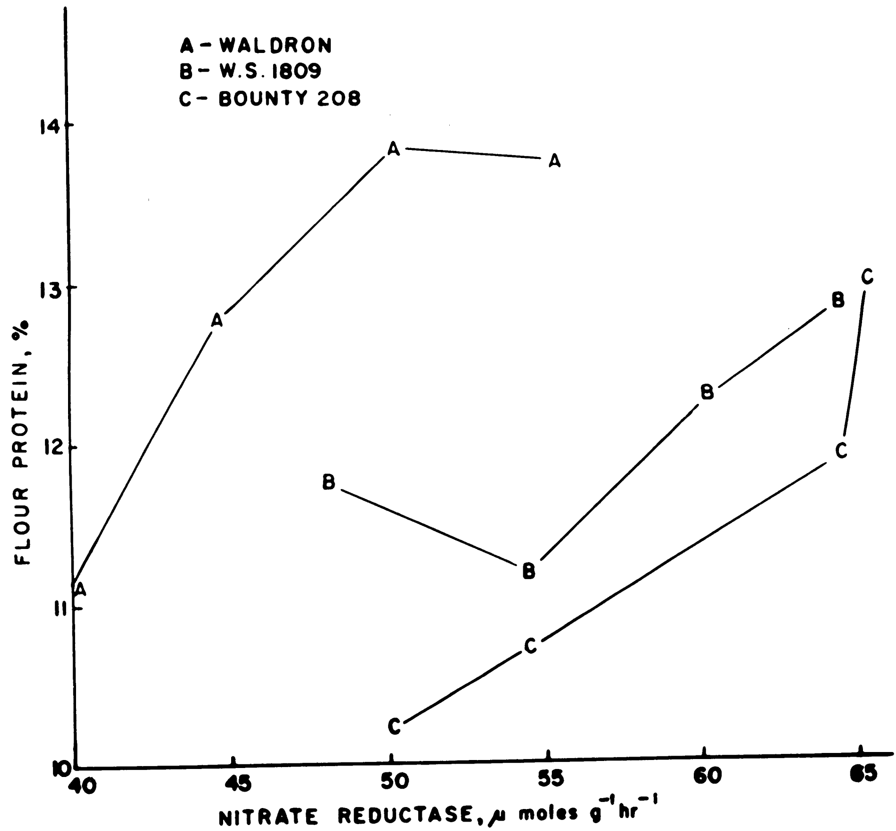

Fig. 2. Relation of the average wheat-leaf tissue nitrate reductase activity to wheat-flour protein content for three HRS wheat varieties. Nitrate reductase data are the average of eight samples collected in triplicate throughout the growing season and are expressed on a dry tissue basis. Protein is on a 14% flour moisture basis.

fertilizer nitrogen rates of 0, 35, 70, and 140 lb N/acre replicated four times. Fertilizer nitrogen, ammonium nitrate, was broadcast May 4. Analysis of soil samples removed from the upper foot on June 8 showed the plots contained 35, 64, 101, and 136 lb N/acre, as NO_3^-, respectively. Two inches of irrigation water were applied by sprinkler on July 9 to give a total of 10 in. of water (rain plus irrigation) for the growing season. The crop also used water stored in the soil prior to planting.

Ten-gram samples of leaf tissues were harvested from one row of each plot and analyzed for nitrate reductase activity at weekly intervals beginning with the two-leaf stage until the plants began to mature and set seed.

Assay Procedure

Nitrate reductase activity of the freeze-dried leaf tissue was determined by the Hageman and Flesher (13) method as modified by Eilrich (14). Tests of fresh and freeze-dried tissue showed no significant enzyme loss due to freeze-drying. Nitrate reductase activities were reported as moles of nitrate produced per hr per g of dry plant tissue. Grain protein analyses were performed according to AACC approved methods (15).

Milling and Baking

Wheat samples were milled to flour on a Buhler pneumatic laboratory mill (type MLU-202). Clean wheat was tempered to 16% moisture 16 hr before milling, and 0.5% moisture was added 5 min before milling. The milling conditions described by Shuey *et al.* (16) were used to produce six streams of flour. All six flour streams were combined to prepare a patent flour. Flour samples were stored under refrigeration at 5° C prior to testing and analysis.

Mixing properties of dough samples were determined by the method of Shuey (17). Mixograms were determined by using 30 g of flour and 20 ml of water. A mixograph spring setting of 10 was used, and all samples were run at a constant weight of flour and volume of water. Mixing scores were determined by comparing the mixing curves for the samples with reference mixogram patterns. A numerical mixing score was assigned to each pattern to classify the curves. A large number indicated stronger mixing characteristics.

A straight-dough bread-baking procedure (17) was used. The baking formula used was as follows: flour, 100%; salt, 2%; sugar, 5%; dried skim milk, 3%; yeast, 3%; shortening, 2%; barley malt flour, 0.1%; and bromate, 10 ppm.

RESULTS AND DISCUSSION

Protein Content

The average flour protein contents of the three wheat varieties *vs.* soil nitrate levels to the 12-in. depth on June 8 are depicted in Fig. 1. The protein content of the wheat generally increased at higher levels of soil nitrate. The response, however, was not equal for all three wheat varieties. Waldron showed a rapid increase in flour protein from 35 to 101 lb N/acre, but failed to increase significantly in protein with further nitrogen fertilization. Furthermore, Waldron was higher in protein content than W.S. 1809 and Bounty 208 for the respective nitrate levels.

TABLE I

Analysis of Variance: Influence of Wheat Varieties and Soil Nitrate Level on Nitrate Reductase and Flour Quality

		Test of Significance										
Source of Variation	**D.F.**	**Nitrate reductase**	**Flour milling yield**	**Flour protein**	**Flour ash**	**Mixing time**	**Baking absorption**	**Loaf volume**	**Bread score**	**Crust color**	**Crumb color**	**Grain texture**
Replication	(R) 3	ns[a]	ns	ns	ns	**	ns	**	*	*	ns	*
Fertilization	(F)	+[b]	ns	**	**	*	ns	**	*	**	ns	ns
Variety	(V) 2	**	**	**	**	**	ns	**	*	**	ns	ns
VXF	6	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[a]Not significant.

[b]The F value was 3.32. An F value of 3.82 is required for significance at the 5% level and 2.81 at the 10% confidence level.

World Seeds 1809 and Bounty 208 had distinctly different flour protein responses to soil nitrate than Waldron. World Seeds 1809 decreased slightly in flour protein at low nitrate levels (35 to 64 lb N/acre), although a rapid increase in flour protein occurred at high soil nitrate levels. Bounty 208 had a slight increase in flour protein at low levels of soil nitrate, with a rapid increase in protein at the two highest nitrate levels.

Unlike Waldron, which decreased in flour protein at high levels of soil nitrate, W.S. 1809 and Bounty 208 continued to increase in flour protein. It appeared that the maximum flour protein content of these two semidwarf wheat varieties had not been reached at the highest soil nitrate level present in the soil to the 12-in. depth. The presence of more than 136 lb N/acre as NO_3^- would have resulted in further increases in flour protein for W.S. 1809 and Bounty 208, but not for Waldron.

Figure 2 shows the relation of wheat-flour protein content to the average seasonal nitrate reductase level of wheat-leaf tissue. Figure 2 indicates the three wheat varieties are quite diverse in their nitrate reductase activities. Waldron appears to reach its maximum protein content at a relatively low level of nitrate reductase activity, while W.S. 1809 and Bounty 208 increase slowly in protein content at relatively high reductase activities. The latter two varieties did not appear to reach a maximum protein level.

Bread and Flour Quality

Analysis of variance data in Table I show that wheat variety and nitrogen fertilization had significant effects on nitrate reductase activity, flour quality, and bread-baking characteristics. Soil nitrate had a highly significant influence on flour protein, flour ash, loaf volume, and crust color, and a significant effect on mixing time and bread score. Since flour protein is generally recognized as contributing to bread quality, the relation of soil nitrate to bread quality was probably due to increased flour protein at high soil nitrate levels. Wheat varieties had a highly significant influence on nitrate reductase activity, flour milling yield, flour protein, flour ash, mixing time, loaf volume, bread score, and crust color. Variety-fertilization interactions were not significant for any of the evaluated fractions.

TABLE II
Linear Correlation Coefficients of Soil Nitrate and Nitrate Reductase *vs.* Flour and Bread Quality[a]

Quality Factor	Correlation Coefficients: Soil nitrate	Correlation Coefficients: Nitrate reductase
Flour protein content	0.60**	0.22
Flour ash content	−0.45**	−0.72**
Mixing time	−0.21	0.20
Mixing score	0.17	0.49**
Bake absorption	0.45**	0.03
Bread loaf volume	0.60**	0.27
Bread crust color	0.33*	0.05
Bread crumb color	0.07	0.18
Bread grain and texture	0.33*	0.25
Nitrate reductase activity	0.79**	...

[a]Data represent four replicates, three varieties, and four soil nitrate levels.

To determine specific relations among soil nitrate level, nitrate reductase activity, and quality characteristics, linear correlation coefficients were calculated. Table II shows that soil nitrate was positively correlated with flour protein, baking absorption, bread loaf volume, crust color, and bread grain and texture, and negatively correlated with flour ash content. Soil nitrate was also highly correlated ($r = 0.79$) with the nitrate reductase activity of wheat-leaf tissue. Table II shows that nitrate reductase activity was positively correlated with mixing score but negatively correlated with flour ash content.

Figure 3 shows that the influence of soil nitrate on bread loaf volume was not equal for the three varieties. World Seeds 1809 increased in loaf volume only at the 101 and 136 lb/acre soil nitrate level. Waldron, on the other hand, showed an almost linear response in loaf volume to each increase in soil nitrate. At the highest nitrate level tested, however, the range in loaf volume among wheat varieties was small (883 to 901 cc). As soil nitrate increased, the loaf volumes of W.S. 1809 and Bounty 208 improved, and were essentially equal to Waldron at high levels of soil nitrate.

The relation of wheat-leaf tissue nitrate reductase activity to bread loaf volume

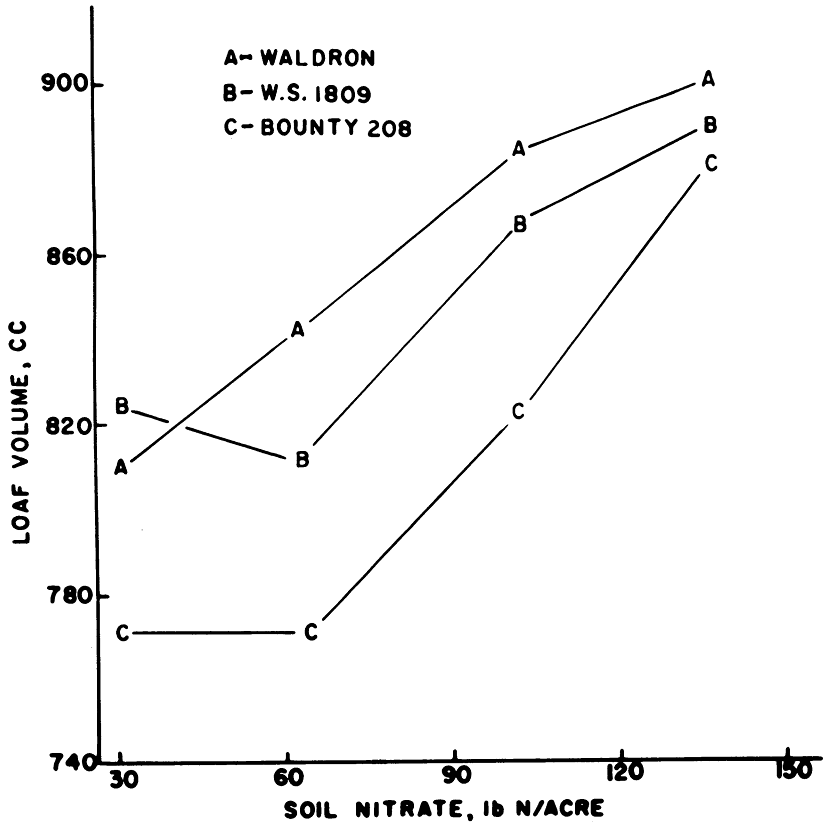

Fig. 3. Influence of soil nitrate levels on bread loaf volume for three wheat varieties. Soil data are expressed in lb N/acre, as nitrate in the top 12 in. of soil on June 8.

is shown in Fig. 4. The similarity in the trends shown in Figs. 3 and 4 demonstrates the close relation of soil nitrate to wheat nitrate reductase activity. Waldron had a nearly linear increase in loaf volume with increasing nitrate reductase.Bounty 208 was lower in loaf volume than Waldron but quite high in nitrate reductase activity. At their respective highest nitrate reductase activities, all three varieties were essentially equal in loaf volume.

CONCLUSION

Wheat-leaf tissue nitrate reductase activity and soil nitrate level showed a significant influence on flour protein content and bread-baking quality of wheat. In replicated field trials of three wheat varieties, Waldron had the highest average protein content and lowest average nitrate reductase activity. At low levels of soil nitrate, Waldron had higher bread loaf volumes and produced a better quality bread than W.S. 1809 and Bounty 208 varieties. However, at high levels of soil

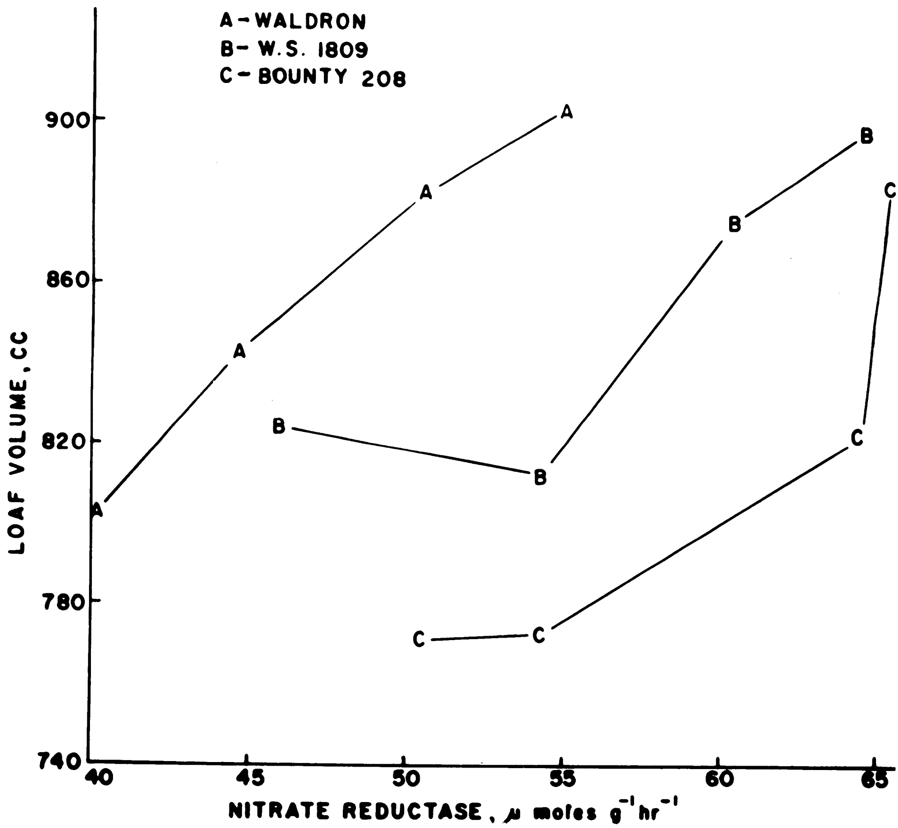

Fig. 4. Relation of wheat-leaf tissue nitrate reductase activity to bread loaf volume. Nitrate reductase data are the average value for eight samples collected in triplicate throughout the growing season and expressed on a dry tissue basis.

nitrate all three wheat varieties produced similar bread of good quality. Nitrate reductase activity in wheat-leaf tissue was correlated with flour ash content and dough-mixing score. However, there was a wide variation among wheat varieties in the relation of quality to nitrate reductase. Consequently, it appeared doubtful that wheat-leaf tissue nitrate reductase concentration would be practical as a test for predicting the flour protein content or baking quality of wheat varieties.

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