PROSPECTS FOR IMPROVING GRAIN PROTEIN CONCENTRATION IN WINTER WHEAT THROUGH PLANT BREEDING

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ABSTRACT

Low protein concentration has been a degrading factor for stubbled-in winter wheat. Genes for improved protein concentration have been reported in several foreign cultivars. This paper outlines the results of plant breeding efforts to incorporate these genes into a Norstar or related background. The interactions of these genes with the environment were also studied in field experiments conducted over a 3 year period in Saskatchewan. Response of grain yield and protein concentration to applied nitrogen was similar for each cultivar studied but the highest yielding genotypes tended to have the lowest protein concentration. Heritability estimates for grain yield, protein concentration and protein yield were fairly high (0.47 to 0.75) but very large environmental effects can severely limit the effectiveness of selection for these traits in winter wheat.

INTRODUCTION

Low grain protein concentration of stubbled-in winter wheat (Triticum aestivum L.) has been a problem in Saskatchewan. Genetic sources of high protein have been reported in foreign cultivars (Goertzen and Goertzen, 1983; Johnson et al., 1985). However, the possibility of transferring such high protein genes to adapted cultivars such as Norstar has not been investigated.

Fowler and de la Roche (1975) reported that the environment had an important effect on protein and yield in spring and winter wheat. Heritability estimates for protein concentration in wheat vary from very low to near 0.8 (Fowler and de la Roche, 1975; Loffler and Busch, 1982; Loffler et al., 1983; Guthrie et al., 1984). Heritability is dependent on the population from which it is calculated and it would be useful to measure the heritability of protein in a population which could be used to improve protein in Norstar or a related winter wheat.

The objectives of this study were to examine the relative effect of the environment and genetics on grain protein concentration in winter wheat and to determine if plant breeding could be used to increase protein concentration in winter wheat adapted to Saskatchewan conditions. Grain yield and protein yield were also studied because of the major influence of yield on protein concentration.

MATERIALS AND METHODS

Varietal Response to Applied Nitrogen

Trials were established to examine five cultivars of winter wheat grown at five rates of nitrogen fertilizer. The trials were located at Saskatoon in 1983-'84 and at Paddockwood and Porcupine Plain in 1985-'86. The cultivars Brawny, Redwin, Norstar, Ulianovka and Fredrick were selected to represent a range in protein concentration. Brawny was replaced by Norwin and Fredrick was replaced by Yorkstar at Porcupine Plain.

A four replicate split plot design with varieties as the main plots and nitrogen rates at the subplots was used in all trials. Ammonium nitrate fertilizer (34-0-0) was broadcast on the plots in early May at rates of 0, 34, 67, 100 and 134 kg of N/ha. Soil samples were collected in spring to determine residual soil nitrogen levels. Grain yield, protein concentration, protein yield, kernel hardness, test weight and kernel weight were determined for each plot. Analyses of variance were used to determine the significance of differences among treatments.

For each cultivar, an inverse polynomial function (Equation 1) was used to describe the response of grain yield and grain protein yield to nitrogen (Fowler et al., 1987).

$$Y = \frac{uN}{N + u/e}\,(1-N/s) \qquad (1)$$

where: Y = predicted grain or grain protein yield (kg/ha)
N = total available nitrogen in kg/ha (residual N + applied N)
s = regression coefficient, a constant which accounts for yield sensitivity to high N levels. Larger s values indicate lower sensitivity.
u = upper limit of yield which could be achieved if no yield sensitivity to high N were to occur.
e = regression coefficient, the initial slope of the nitrogen response.

The values for s and e were set at 903 and 61.7 for grain yield and 949 and 5.2 for grain protein yield. These values were obtained from least squared estimates (SAS, 1985) of all three coefficients using data from 28 Norstar winter wheat fertilizer trials. The coefficient u was then obtained using least squares estimation. The maximum grain and grain protein yields (Ymax) and the nitrogen level at which the maximum could be produced (Nmax) were calculated as described by Fowler et al. (1987).

The response of grain protein concentration of each cultivar to applied nitrogen was best described by the Gompertz equation (Fowler et al., 1987):

$$P = M + A\exp[-B\exp(-KN)] \qquad (4)$$

where: P = predicted protein concentration (%)
M = minimum protein concentration (%)
M + A = asymptotic protein concentration achieved at high N levels.
B = determines the N level at which protein concentration reaches M + 0.5A.
K = coefficient that determines the rate P increases to M + A.
N = total available nitrogen (kg/ha).

Fowler et al. (1987) estimated that the minimum protein concentration was 8.5% (14% moisture basis). This value was converted to a dry weight basis (9.9%) and used as a constant in the equation. The coefficient K was also found to be constant at 0.02302. The coefficients A and B were determined by least squares estimation (SAS, 1985).

Variability in Pure Stands of Winter Wheat

Uniformity trials were established in solid seeded fields of Norstar winter wheat at Clair in 1984-'85 and 1985-'86. Sites were chosen to represent uniform areas of the field and were treated uniformly prior to harvest. Single row plots were harvested using a randomized completed block design with four replicates and 120 plots per replicate. Grain yield, protein concentration, protein yield, test weight, kernel weight, and kernel hardness were determined for each plot.

Plots ranging in size from 2 to 8 row plots were obtained by combining the data obtained from single row plots. Analyses of variance were conducted using randomly assigned 'treatment' values. For each plot size, the method described by Cochran and Cox (1957) was used to estimate the number of replications required to provide an 80% probability of detecting differences among plots at the 5% level of significance using a two tailed t-test. It was assumed that four treatments would be compared.

Broad Sense Heritability

Six winter wheat cultivars representing a range in grain protein concentration were used to develop material for genetic studies. The cultivars were Norstar, Alabaskaja, Ulianovka and Vona, Plainsman V and Yorkstar. Simple crosses, backcrosses, 3-way crosses and double crosses were made among these cultivars. Lines derived from these crosses were grown at Saskatoon and Outlook in 1983-'84, at Outlook in 1984-'85 and at Paddockwood and Porcupine Plain in 1985-'86. A randomized complete block design with 2 or 3 replicates was used at each location. Grain yield, protein concentration, protein yield, test weight, kernel

Table 1. Analysis of variance and expectations of mean squares with environments random and genotypes fixed.

Source of Variation	df[1]	Expected Mean Squares
Environments (E)	e-1	$\sigma^2 + g\sigma^2_{r(e)} + rg\sigma^2_e$
Replicates(Envt) [R(E)]	e(r-1)	$\sigma^2 + g\sigma^2_{r(e)}$
Genotypes (G)	g-1	$\sigma^2 + r\sigma^2_{eg} + er\phi_g$
E x G	(e-1)(g-1)	$\sigma^2 + r\sigma^2_{eg}$
Error	e(r-1)(g-1)	σ^2

[1]df = degrees of freedom

weight and kernel hardness were determined for each plot.

Analyses of variance were conducted and all locations used over the three year period were combined. The components of variance for each trait were calculated using the expectations of mean squares listed in table 1. Heritability was estimated for each trait using equation 5 and 90% confidence intervals were calculated according the method of Knapp et al. (1985). Heritability was also estimated using data from the varietal response trials. All three trials were combined using only the fertilizer rate at which yield and protein concentration appeared to be maximized.

$$h^2 = \frac{\phi_g}{\phi_g + \sigma^2_{ge}/e + \sigma^2/re} \qquad (5)$$

RESULTS AND DISCUSSION

Varietal Response to Applied Nitrogen

The three locations used for these trials varied considerably. The Saskatoon site (1983-'84) had a residual soil nitrogen level of 103 kg/ha. There was 46 kg of residual N/ha at Paddockwood (1984-'85). The trial at Porcupine Plain (1985-'86) was situated on summerfallow and the soil had a high organic matter content and a residual nitrogen level of 311 kg/ha.

Analyses of variance indicated that there were significant differences among the cultivars studied for grain yield, protein concentration and protein yield (Table 2). Nitrogen fertilizer rate had a significant effect on all three measurements at Paddockwood and on grain yield and protein yield at Porcupine Plain. Only grain protein concentration was significantly affected by changing nitrogen levels at the Saskatoon site. There was a significant cultivar by nitrogen rate interaction for

Table 2. Summary of the results of the analyses of variance for each location.

Trait	Location	Significance of Treatment Differences		
		Cultivar	Rate	Cultivar x Rate
Grain Yield	Saskatoon	**[1]	NS	NS
	Paddockwood	**	*	NS
	Porcupine Plain	**	**	NS
% Protein	Saskatoon	**	**	**
	Paddockwood	**	**	NS
	Porcupine Plain	**	NS	*
Protein Yield	Saskatoon	*	NS	NS
	Paddockwood	*	**	NS
	Porcupine Plain	**	**	NS

[1]NS=not significant at P=0.05; *=significant at P=0.05; **=significant at P=0.01.

protein concentration at Saskatoon and Porcupine Plain but the cultivars did not change in rank over the range of nitrogen levels used in these trials. Bole and Dubetz (1986) observed a significant cultivar by nitrogen interaction for yield but not for protein concentration of soft white spring wheat. No change in rank of cultivars was observed. Kosmolak and Crowle (1980), in field experiments with spring wheat, reported that there were no significant cultivar by nitrogen level interactions for either grain yield or protein concentration.

The predicted yield response curves for each cultivar (calculated using equation 1) are illustrated in figure 1 using only the nitrogen levels found at each location. The curves for each cultivar increased with increased nitrogen at Paddockwood. However, they were relatively constant at Saskatoon and decreased slightly at Porcupine Plain. Each of these locations represent a different part of the overall response curve. The low yields observed at the Saskatoon site were due to a very dry growing season.

Norstar had the highest grain yields at all locations and Brawny had the lowest yields where it was included (Figure 1). All cultivars had similar yield responses but they differed in maximum predicted yields and in the amount of nitrogen required to produce the maximum (Table 3).

The predicted response curves for grain protein concentration (calculated by the Gompertz equation) are shown in figure 2. Once again, the curves at Paddockwood illustrated the increasing phase of the relationship while the curves at

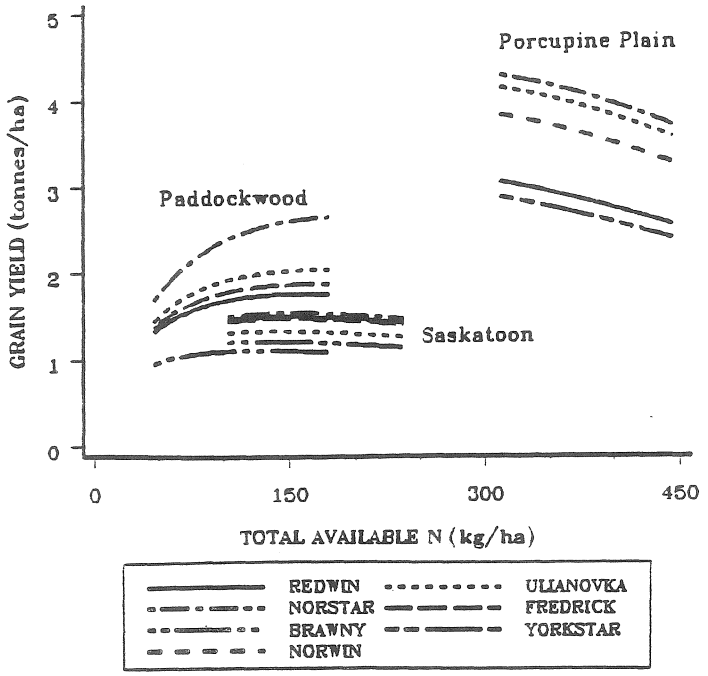

Figure 1. Predicted nitrogen response curves for grain yield of each cultivar at each location.

Table 3. Estimates of the coefficient u, its standard error, the maximum predicted grain yield (Ymax) and the nitrogen level at which the maximum yield could be produced (Nmax) for each cultivar at each location.[1]

Location	Cultivar	u	Standard Error	Ymax kg/ha	Nmax kg N/ha
Saskatoon (1983-'84)	Redwin	2245	63.4	1507	148
	Ulianovka	1944	42.5	1341	140
	Norstar	2328	65.6	1551	151
	Fredrick	2189	93.4	1476	147
	Brawny	1733	73.1	1220	134
Paddockwood (1985-'86)	Redwin	2730	153.4	1760	161
	Ulianovka	3291	68.8	2034	172
	Norstar	4701	172.8	2650	197
	Fredrick	2956	138.6	1872	166
	Brawny	1555	126.6	1115	128
Porcupine Plain (1985-'86)	Redwin	6130	399.0	3194	216
	Ulianovka	9430	573.5	4232	249
	Norstar	9923	643.3	4367	253
	Yorkstar	5670	551.7	3027	211
	Norwin	8380	461.7	3929	240

[1]The coefficient u was determined using equation 1 with e = 61.7 and s = 903.

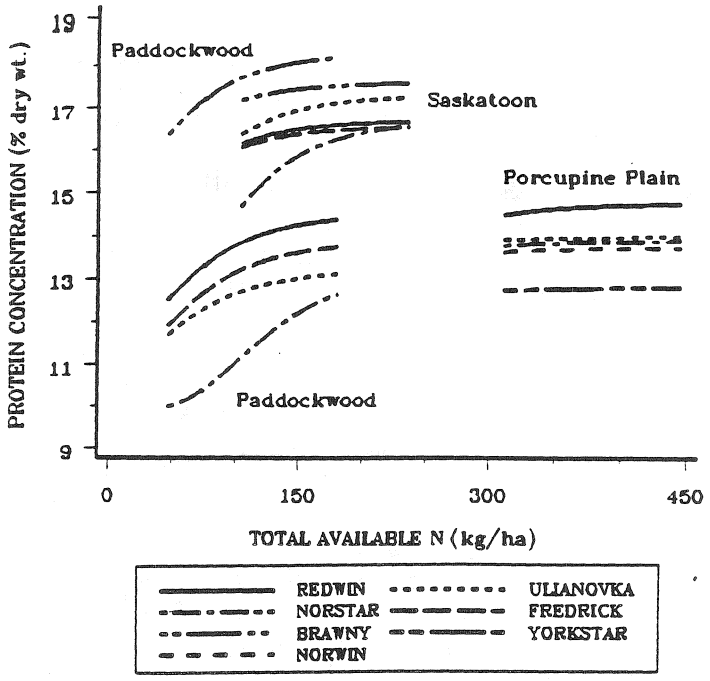

Figure 2. Predicted nitrogen response curves for grain protein concentration of each cultivar at each location.

Table 4. Estimates of the coefficients A and B from the Gompertz equation (Equation 4) and the standard errors of these estimates for each cultivar at each location.[1]

Location	Cultivar	A	Standard Error	B	Standard Error
Saskatoon (1983-'84)	Redwin	6.75	0.08	0.87	0.26
	Ulianovka	7.34	0.10	1.42	0.31
	Norstar	6.72	0.18	3.73	0.70
	Fredrick	6.62	0.10	0.83	0.32
	Brawny	7.64	0.19	0.57	0.53
Paddockwood (1985-'86)	Redwin	4.56	0.15	1.64	0.25
	Ulianovka	3.27	0.15	1.74	0.36
	Norstar	3.19	0.53	10.25	3.63
	Fredrick	3.94	0.34	1.97	0.71
	Brawny	8.34	0.16	0.75	0.12
Porcupine Plain (1985-'86)	Redwin	4.80	0.14	72.87	76.73
	Ulianovka	4.02	0.07	19.66	5.27
	Norstar	3.93	0.05	33.34	4.08
	Yorkstar	2.86	0.15	28.81	15.96
	Norwin	3.78	0.07	30.58	5.98

[1] M = 9.9 and K = 0.02302.

Saskatoon and Porcupine Plain were fairly level.

An examination of the B values (which determine when the protein concentration reaches the midpoint between the minimum and maximum values) shows that Norstar had a much larger B value than any other cultivar at Saskatoon and Paddockwood (Table 4). With its higher yields, it took more nitrogen to begin increasing protein concentration for Norstar than for the other cultivars. The B values at Porcupine Plain were very large and difficult to interpret since the nitrogen levels were high and the curves were past the increasing phase. Comparison of the maximum predicted protein concentration (M + A) of each cultivar provides an indication of the genetic variability present. The high protein concentration of Brawny, which was caused by low yields, inflated the estimate of genetic variability at Paddockwood. The range in maximum protein values (12.8% to 14.7%) at Porcupine Plain may be a more realistic estimate of genetic variability. The maximum predicted protein concentration of Norstar was 13.8% so there is potential to increase the protein concentration of Norstar by as much as 1% using these varieties in a breeding program.

Figure 3 illustrates the predicted response curves for grain protein yield. These curves are similar to the yield curves and the cultivars occupy the same relative positions. Norstar had the best protein yields and the highest maximums (Table 5), even though it had very low protein concentrations. Norstar also required the most nitrogen to reach its maximum (Table 5).

The functions used to predict grain yield, protein concentration and protein yield fit the actual data very well (Figure 4). Coefficients of determination for actual versus predicted values were 0.95, 0.99 and 0.96 for grain yield, protein concentration and protein yield, respectively. Fowler et al. (1987) also found that these equations were very useful in predicting nitrogen responses of winter wheat. In contrast, Bole and Dubetz (1986) used a quadratic equation to predict yield and protein responses of several soft white spring wheat cultivars. However, the quadratic equation does not account for the initial lag phase in nitrogen response of protein concentration. Also, the value for available nitrogen appears twice in the quadratic equation and this is difficult to interpret biologically.

Variability in Pure Stands of Winter Wheat

The uniformity trials grown in 1984-'85 and 1985-'86 illustrated the variability that can exist within a solid seeded stand of Norstar winter wheat. Figure 5 shows the variation in grain yields from plot to plot. There was a large amount of variation when plots were made up of single rows. By increasing the plot width to five rows, the variability was reduced considerably but there was still some variation. Detecting a difference of 600 kg/ha is possible if plots of three rows or greater are used (Figure 6). However, yields of adapted lines often differ by less than 600 kg/ha. Except for very large differences, using one row plots would require too many replications to be useful in detecting yield differences.

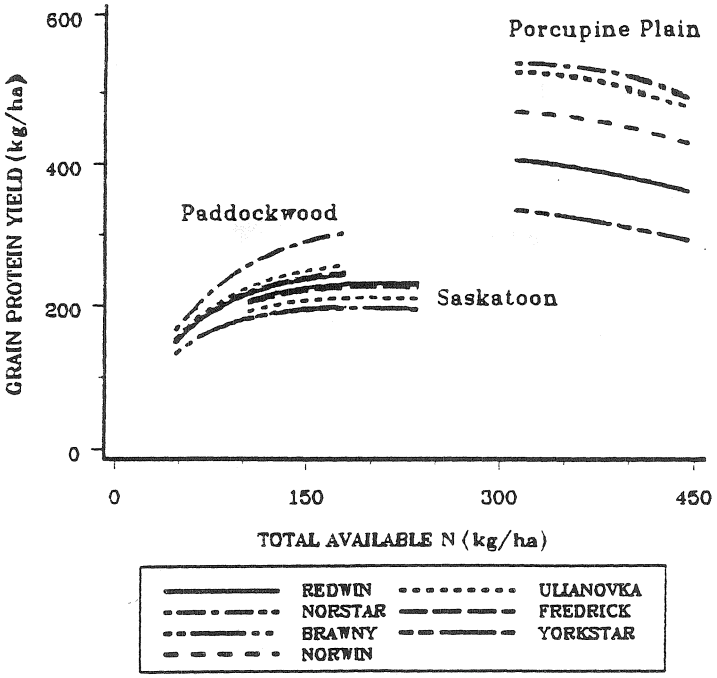

Figure 3. Predicted nitrogen response curves for grain protein yield of each cultivar at each location.

Table 5. Estimates of the coefficient u, its standard error, the maximum predicted grain protein yield (Ymax) and the nitrogen level at which the maximum protein yield could be produced (Nmax) for each cultivar at each location.[1]

Location	Cultivar	u	Standard Error	Ymax kg/ha	Nmax kg N/ha
Saskatoon (1983-'84)	Redwin	406.9	11.8	231	205
	Ulianovka	357.9	12.6	210	196
	Norstar	404.8	10.8	230	205
	Fredrick	392.1	19.2	225	203
	Brawny	326.1	12.8	196	189
Paddockwood (1985-'86)	Redwin	438.2	22.2	244	211
	Ulianovka	475.4	5.7	258	217
	Norstar	617.4	35.6	309	237
	Fredrick	448.6	21.8	248	213
	Brawny	329.4	20.9	198	190
Porcupine Plain (1985-'86)	Redwin	952.7	73.1	406	272
	Ulianovka	1474.5	87.3	519	308
	Norstar	1544.2	84.8	531	311
	Yorkstar	712.6	59.4	339	249
	Norwin	1234.3	70.9	471	293

[1]The coefficient u was determined using equation 1 with e = 5.2 and s = 949.

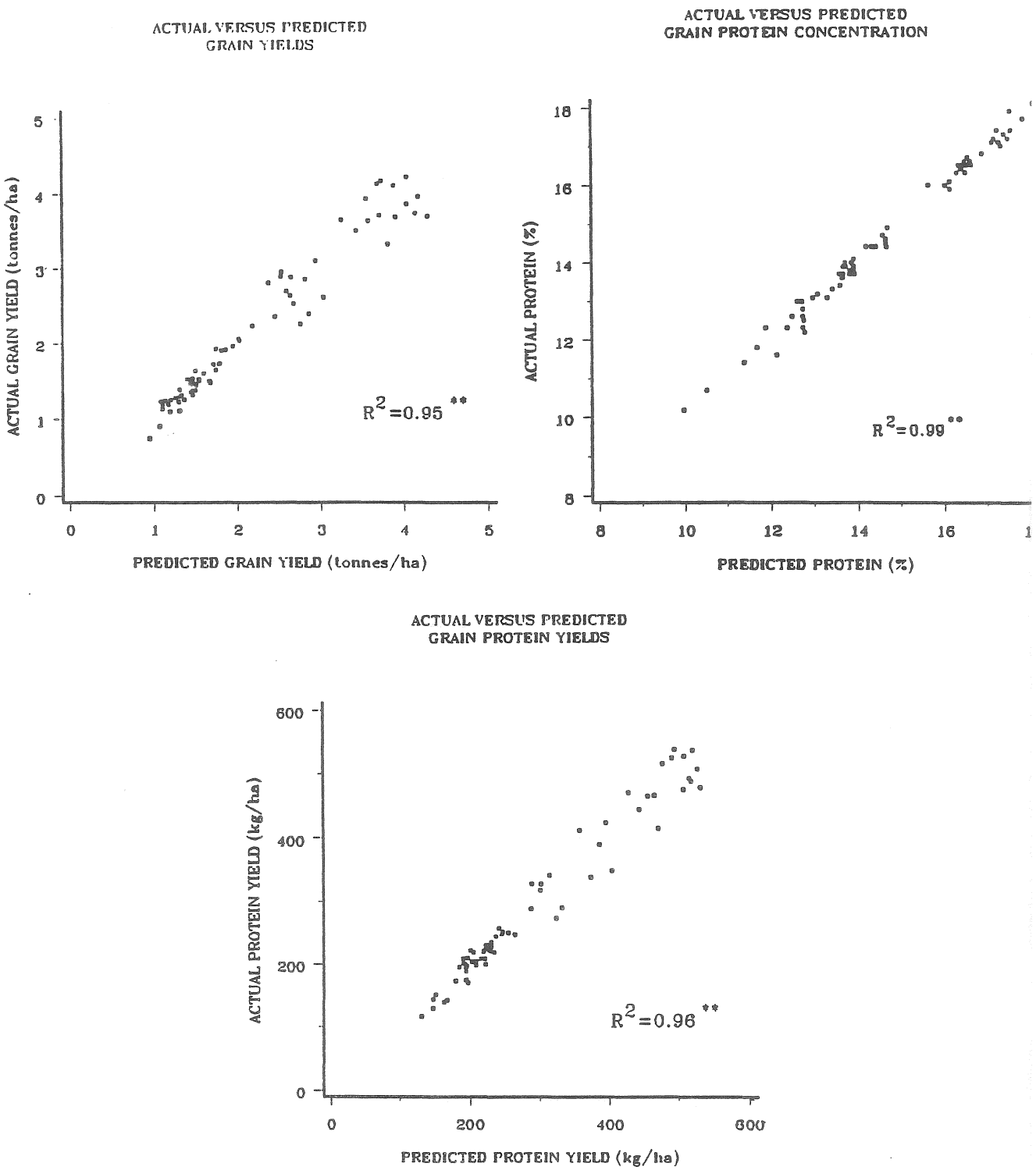

Figure 4. Actual versus predicted grain yield, protein concentration and protein yield.

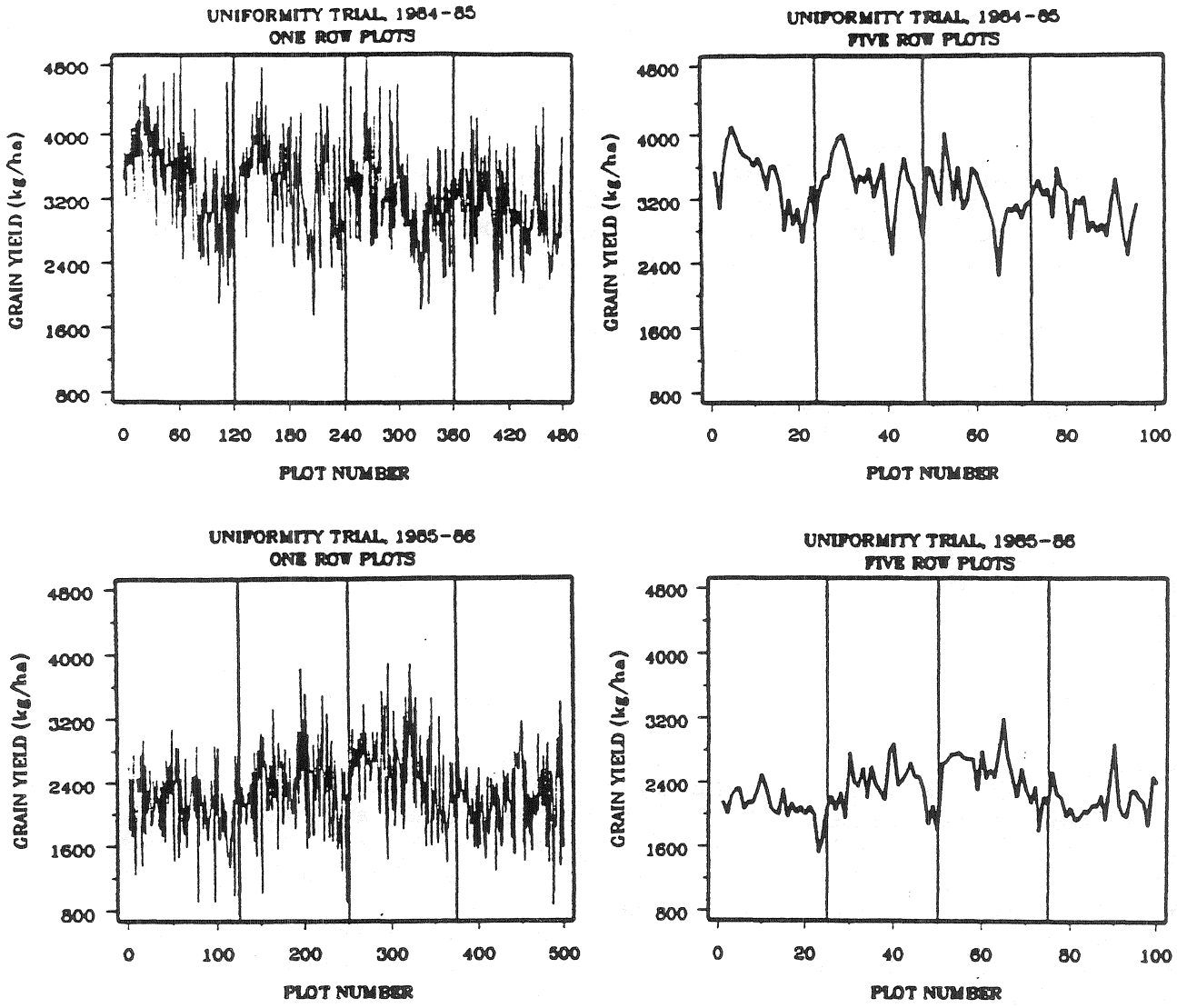

Figure 5. Grain yields of Norstar winter wheat in the 1984-'85 and 1985-'86 uniformity trials.

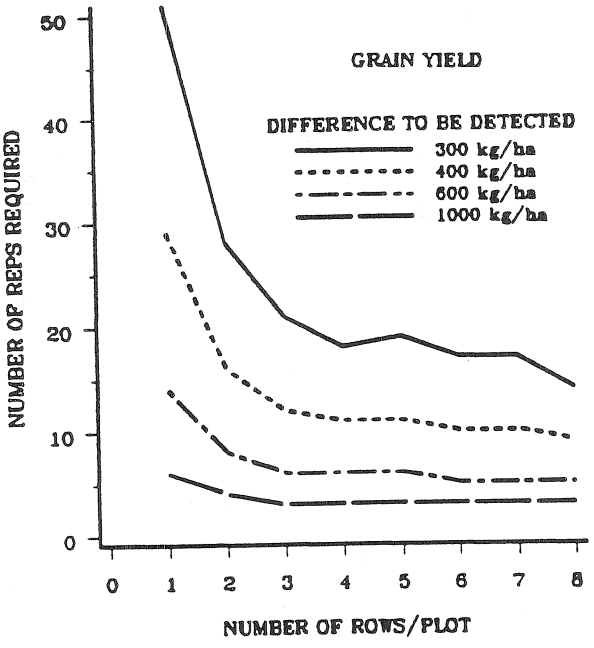

Figure 6. The number of replicates(reps) required to detect specific differences in grain yield for each plot size.

Typically, selection for yield in a breeding program is done on plots of 3 or more rows.

The data for grain protein concentration show much less variability than grain yield (Figure 7). Once again, an increase in plot size to 5 row plots decreased the variability though differences were still found from plot to plot. The number of rows per plot was not as important in determining the number of replicates required to detect differences as it was for grain yield (Figure 8). One row plots required a greater number of replications in most cases but there was less difference among 2 to 8 row plots. However, the differences detected using reasonable numbers of replicates (6 replicates for a 1.0% difference) were large and would not be common in a breeding program.

Grain protein yields appeared to be very similar to grain yields with respect to variability from plot to plot (Figure 9). The use of five row plots reduced variability but much variation was still observed. The number of replicates required to detect specific differences in grain protein yield increased sharply when plot widths were reduced to one or two rows (Figure 10). Plots should consist of 4 or more rows and differences of less than 100 kg/ha could not be detected without large numbers of replicates.

Though the location chosen for these uniformity trials may have been more variable than many sites, the data serve to emphasize the need for plots of three or more rows and large numbers of replicates when selecting for yield and protein in winter wheat. Since winter wheat is seeded into stubble, variability from the previous crop can have a strong influence and trials can be more variable than comparable trials grown on summerfallow.

Broad Sense Heritability

The components of variance method was used to calculate heritability for grain yield, protein concentration, protein yield and kernel hardness using data from the single lines and from the varietal response to nitrogen trials. Five components of variance were calculated: the environmental component (envt), the genetic component (gen), the component due to genetic by environment interaction (g x e), the component due to replication within each environment (rep(e)) and the error component (Figures 11 and 12). There was an extremely large environmental component for all traits. The genetic component for grain yield, protein concentration and protein yield was small. Fowler and de la Roche (1975), working with spring and winter wheat, also observed a large environmental effect for yield, protein concentration and protein related parameters.

Heritability provides a measure of the proportion of genetic variability that is transferred from one generation to the next. Broad sense heritability estimates for the variety trials had very large confidence intervals because only five varieties were used to calculate heritability (Table 6). The heritability of protein concentration was estimated to be 0.60 or 0.64 (Table 6).

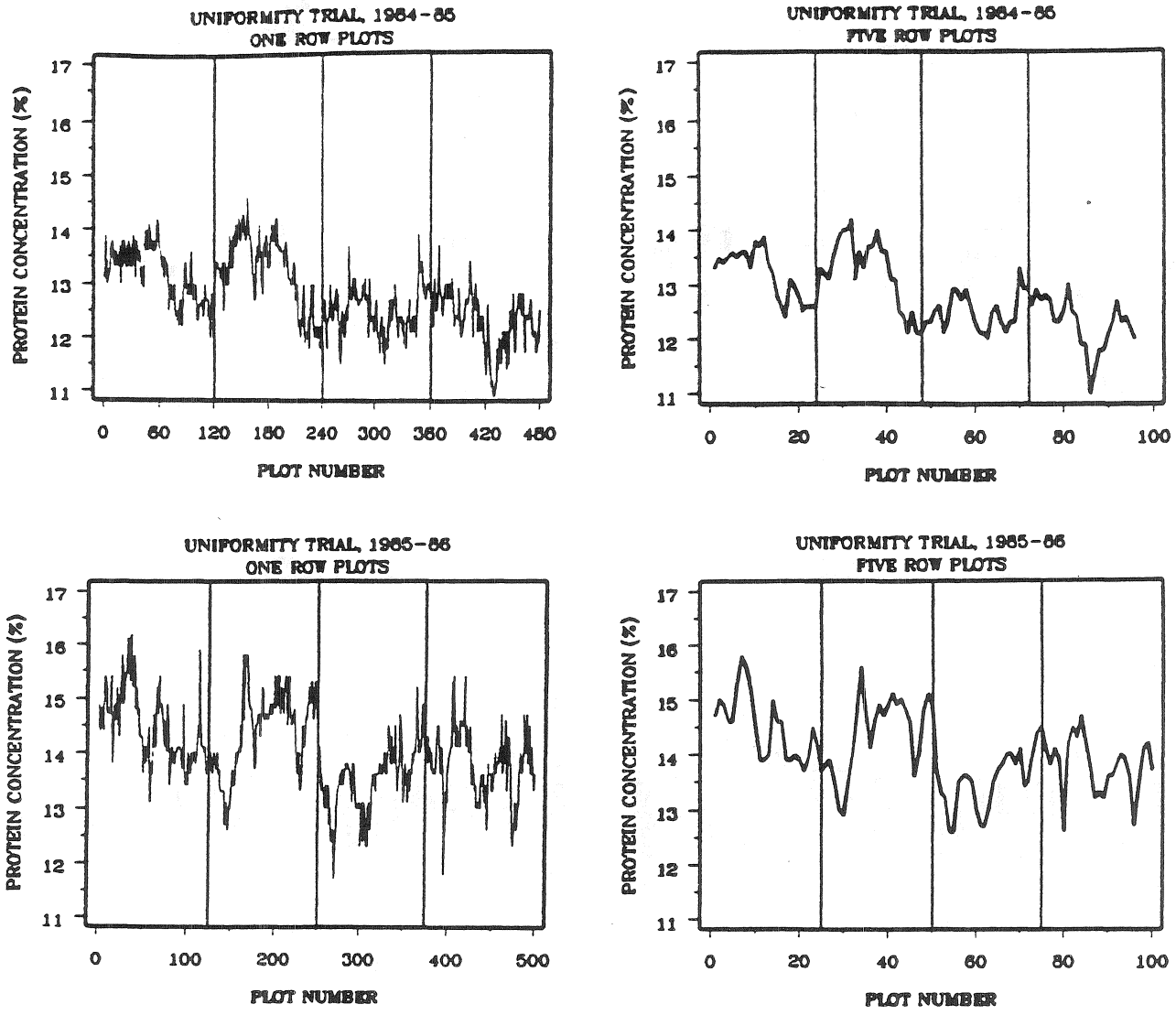

Figure 7. Grain protein concentration of Norstar winter wheat in the 1984-'85 and 1985-'86 uniformity trials.

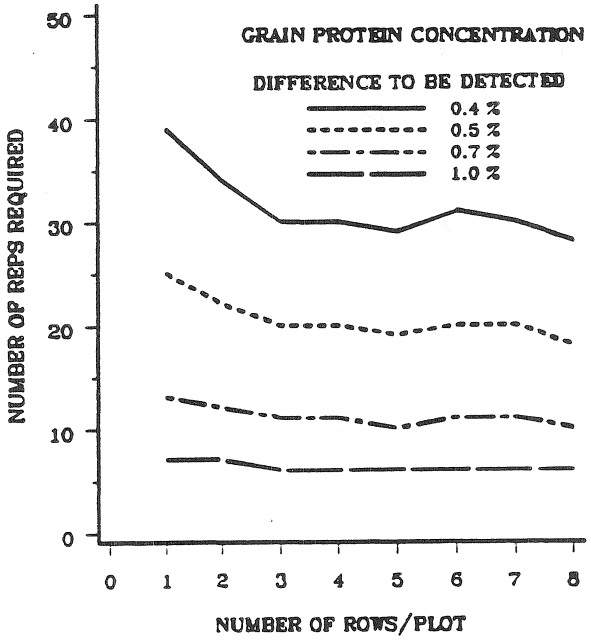

Figure 8. The number of replicates (reps) required to detect specific differences in grain protein concentration for each plot size.

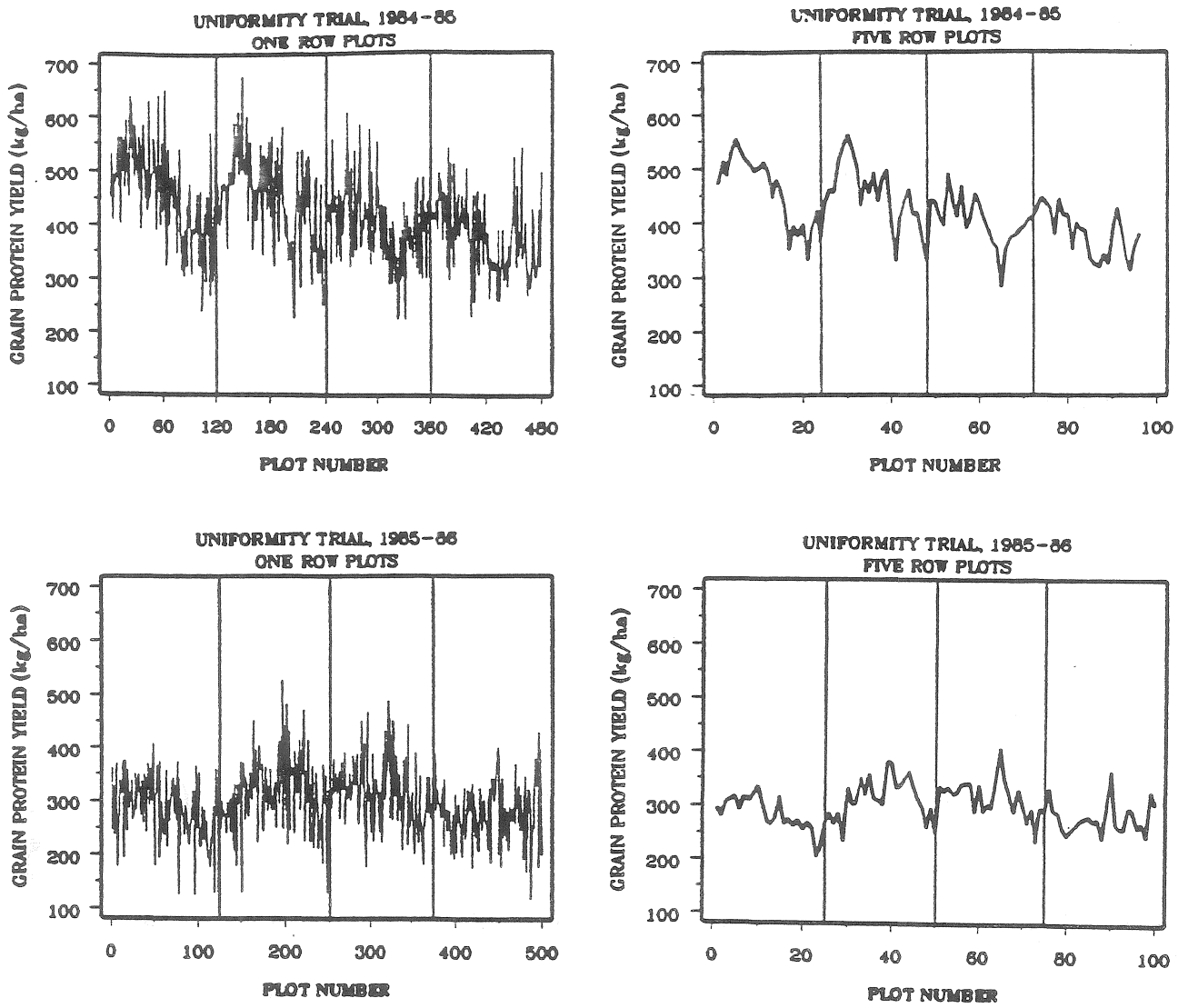

Figure 9. Grain protein yields of Norstar winter wheat in the 1984-'85 and 1985-'86 uniformity trials.

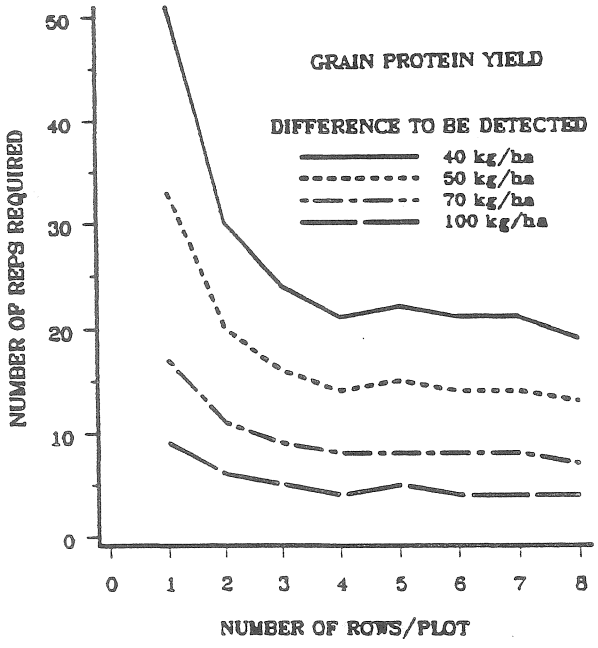

Figure 10. The number of replicates (reps) required to detect specific differences in grain protein yield for each plot size.

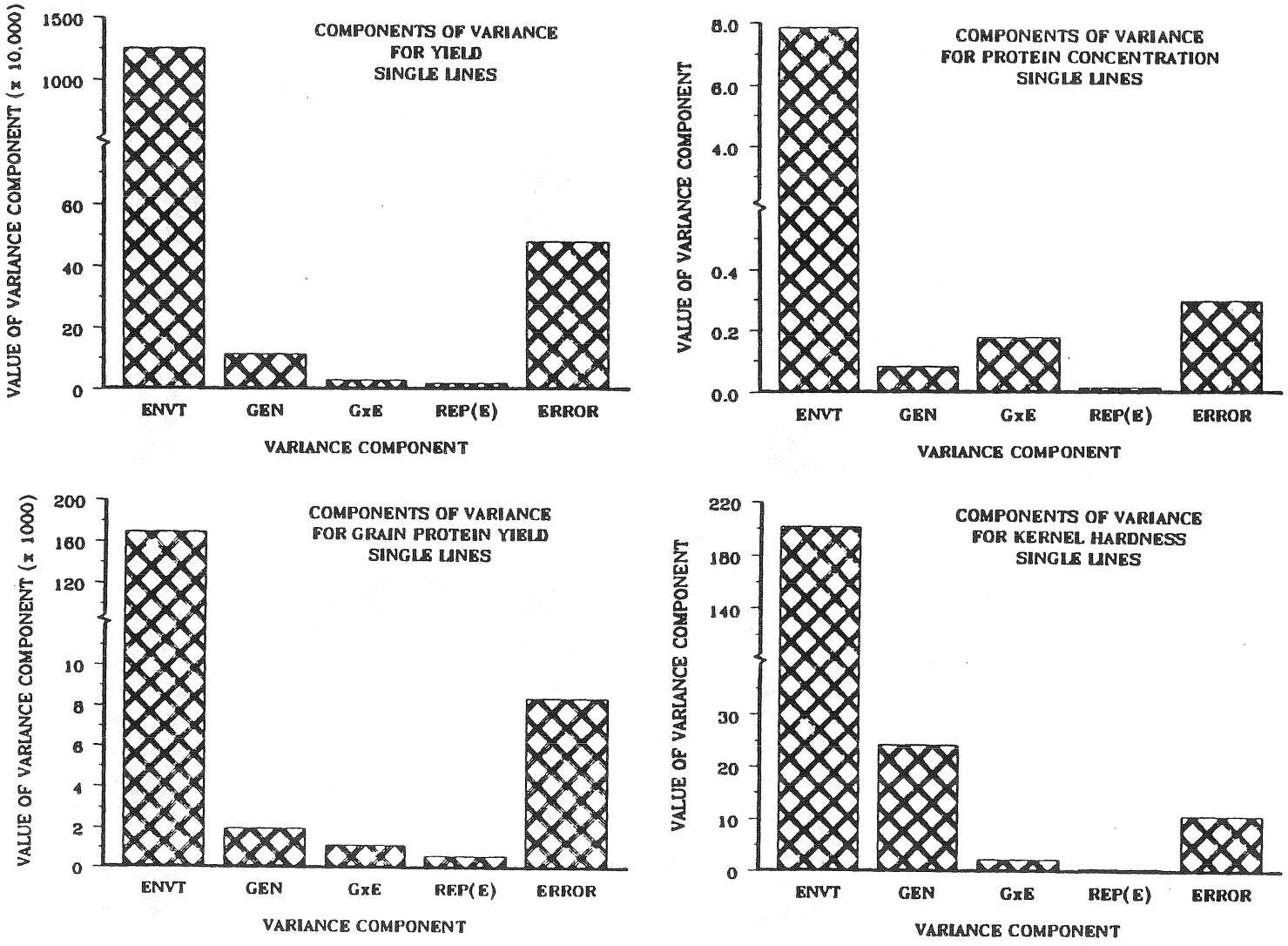

Figure 11. The size of the components of variance for yield, protein concentration, protein yield and kernel hardness of the single lines.

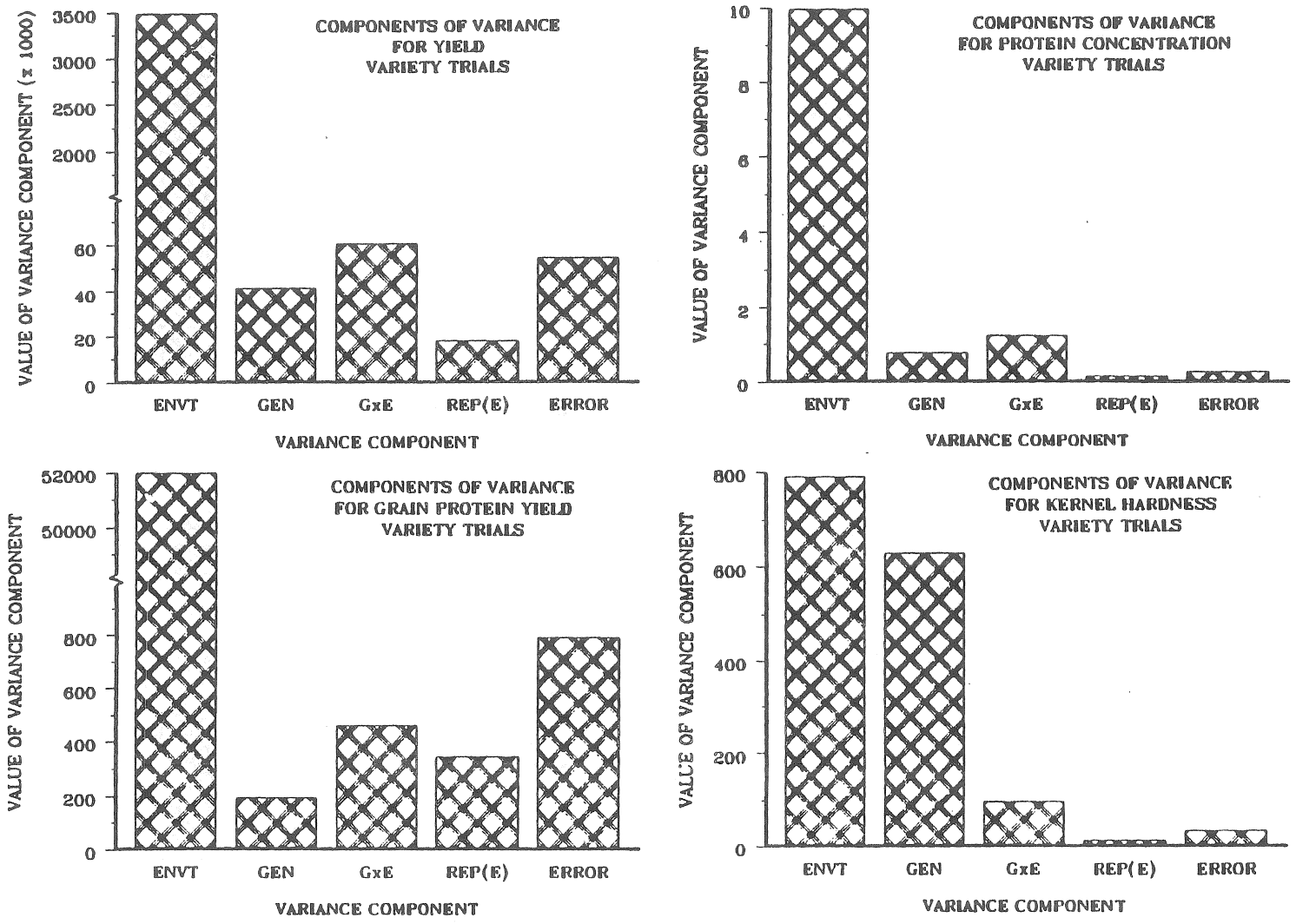

Figure 12. The size of the components of variance for yield, protein concentration, protein yield and kernel hardness of the variety trials.

Table 6. Estimates of broad sense heritability of grain yield, protein concentration, protein yield and kernel hardness.

Trait	Heritability Estimates	
	Variety Trials	Single Lines
Grain Yield	0.52 (-1.15 to 0.92)[1]	0.75 (0.58 to 0.86)
% Protein	0.64 (-0.64 to 0.94)	0.60 (0.32 to 0.78)
Protein Yield	0.47 (-1.43 to 0.91)	0.72 (0.52 to 0.85)
Kernel Hardness	0.95 (0.76 to 0.99)	0.95 (0.92 to 0.97)

[1]Values in brackets are 90% confidence intervals for the heritability estimates.

Guthrie et al. (1984) reported similar values of realized heritability estimates of grain protein concentration in winter wheat, though some estimates were lower than those reported in the present study. Other researchers (Loffler and Busch, 1982; Loffler et al., 1983) reported larger estimates for heritability of protein concentration in spring wheat, with values ranging from 0.75 to 0.85. The estimates obtained in this study (0.60 and 0.64) indicate that some progress should be possible when selecting for protein concentration in winter wheat. However, progress will be limited by the small amount of genetic variability present. The range in maximum protein values in the varietal response trials indicated that genetic variability for this trait ranged from 1 to 2 percentage units. This, then, is the maximum change in protein concentration that would be possible using these varieties.

Heritability estimates for grain yield were 0.52 and 0.75 (Table 6). Loffler and Busch (1982) estimated the heritability of grain yield in spring wheat to be 0.70 to 0.78. These values are similar to the upper value obtained in this study. Loffler et al. (1983) estimated heritability of yield in spring wheat to be 0.33 to 0.39 and Guthrie et al. (1984) reported estimates that were even lower. In all three studies, the estimates of heritability of grain yield were lower than those of protein concentration. This also occurred with the variety trials of the present study. However, for the single lines, protein concentration appeared to be less heritable than yield but the confidence intervals for each overlapped.

Estimates of heritability of grain protein yield (0.47 and 0.72) were lower than those of grain yield (Table 6). Loffler and Busch (1982) also found that grain protein yield had a lower heritability estimate than grain yield. This indicates that simultaneous improvement in both yield and protein may be more difficult than improvement in yield alone.

Kernel hardness was much more heritable than the three other

traits examined in this study and its heritability was estimated to be 0.95 for both the variety trials and single lines (Table 6). Fowler and de la Roche (1975) estimated heritability of kernel hardness in spring and winter wheat to be 0.94 and it was also much more heritable than yield or protein. Sampson et al. (1983) reported heritability estimates that ranged from 0.55 to 0.92 for kernel hardness in spring wheat.

SUMMARY

Winter wheat cultivars had similar responses to applied nitrogen, though the cultivars differed in the magnitude of the response. Cultivars with the highest grain yields tended to have the lowest protein concentrations so there may be a problem with an inverse relationship between yield and protein concentration.

Variability within a solid seeded stand of winter wheat was large. More variability was observed for grain yield and protein yield than for protein concentration. The use of plots with three or more rows and large numbers of replicates is recommended for these traits.

The environmental component was the largest variance component for each trait and it may limit the effectiveness of selection for yield, protein concentration and protein yield. Heritability estimates for these traits were fairly high indicating that some progress should be possible though progress may be limited by the amount of genetic variability present. Kernel hardness was highly heritable and least affected by the environment so changes in this trait through plant breeding should not be as difficult.

ACKNOWLEDGEMENTS

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REFERENCES

Bole, J.B. and Dubetz, S. 1986. Effect of irrigation and nitrogen fertilizer on the yield and protein concentration of soft white spring wheat. Can. J. Plant Sci. 66:281-289.

Cochran, W.G. and Cox, G.M. 1957. Experimental Designs. John Wiley and Sons, Inc., New York. 611p.

Fowler, D.B., Brydon, J. and Baker, R.J. 1987. Optimizing nitrogen fertilizer response by winter wheat and rye. In Proc. 1987 Soils and Crops Workshop. University of Saskatchewan, Saskatoon, Sask. pp.70-99.

Fowler, D.B. and de la Roche, I. A. 1984. Wheat quality evaluation. 3. Influence of genotype and environment. Can. J. Plant Sci. 55:263-269.

Goertzen, K.L. and Goertzen, B.L. 1983. Breeding for high protein. In D.B. Fowler, L.V. Gusta, A.E. Slinkard and B.A. Hobin, eds. New Frontiers in Winter Wheat Production. proc. Western Can. Winter Wheat Conference. University of Saskatchewan Printing Services, Saskatoon, Sask. pp.123-135.

Guthrie, D.A., Smith, E.L. and McNew, R.W. 1984. Selection for high and low grain protein in six winter wheat crosses. Crop Sci. 24:1097-1100.

Johnson, V.A., Mattern, P.J., Peterson, C.J. and Kuhr, S.L. 1985. Improvement of wheat protein by traditional breeding and genetic techniques. Cereal Chem. 62:350-355.

Knapp, S.J., Stroup, W.W. and Ross, W.M. 1985. Exact confidence intervals for heritability on a progeny mean basis. Crop Sci. 25:192-194.

Kosmolak, F.G. and Crowle, W.L. 1980. An effect of nitrogen fertilization on the agronomic traits and dough mixing strength of five Canadian hard red spring wheat cultivars. Can. J. Plant Sci. 60:1071-1076.

Loffler, C.M. and Busch, R.H. 1982. Selection for grain protein, grain yield, and nitrogen partitioning efficiency in hard red spring wheat. Crop Sci. 22:591-595.

Loffler, C.M., Busch, R.H. and Wiersma, J.V. 1983. Recurrent selection for grain protein percentage in hard red spring wheat. Crop Sci. 23:1097-1101.

Sampson, D.R., Flynn, D.W. and Jui, P.Y. 1983. Genetic studies on kernel hardness in wheat using grinding time and near infrared reflectance spectroscopy. Can. J. Plant Sci. 63:825-832.

SAS Institute Inc., 1985. SAS User's Guide: Statistics. Version 5 edition. SAS Institute Inc., Cary, NC., USA. pp. 575-606.