# NOTE

# Variation for Starch Concentration in Spring Wheat and Its Repeatability Relative to Protein Concentration<sup>1</sup>

PIERRE HUCL<sup>2</sup> and RAVINDRA N. CHIBBAR<sup>3</sup>.

Cereal Chem. 73(6):756–758

A major objective of Canadian spring wheat breeding programs has been to increase grain protein concentration and quality of cultivars released for commercial production. With the recent discovery of starch mutants in wheat (Yamamori et al 1994), and their association with specific noodle-making quality (Miura and Tanii 1994) emphasis on manipulating the starch composition and quantity of Canadian wheats is likely to gain importance. Furthermore, the recent development of fully waxy endosperm wheat (Nakamura et al 1995) may create new opportunities for the utilization of wheat starch in the food processing industry. High starch content cultivars may become desirable for the production of grain ethanol and biodegradable packaging materials (Wasserman et al 1995). With the exception of soft white wheats, most Canadian wheat cultivars have been selected for elevated protein concentration with less emphasis on selection for increased starch concentration. Selection of wheat genotypes with increased starch concentration will require simple, yet repeatable methods to identify high-starch genotypes. There is a lack of published information regarding the repeatability of starch content measurement across wheat cultivars and environments. The objective of this study was to determine the variation in, and repeatability of, grain starch concentration relative to that of grain protein concentration in spring wheat.

# MATERIALS AND METHODS

Thirty-three spring wheat (*Triticum aestivum* L.) cultivars (Table I) were evaluated for total starch and protein concentration over four (Experiment 1) or three (Experiment 2) years. Experiments were conducted at Saskatoon, SK, Canada. The 33 cultivars evaluated represented four market classes of Canadian spring wheat as well as a number of foreign cultivars varying in kernel texture and color. The cultivars of Experiment 1 were part of a study on sprouting resistance described in Hucl (1994). Five cultivars (Katepwa, Columbus, CDC Teal, Roblin, and Genesis) were common to both experiments.

A randomized complete block design with two replicates was used each year. Plots consisted of three or five rows spaced 20 cm apart or four rows spaced 30 cm apart. The row length was 1.8, 2.4, or 3.7 m. The soil type was a Sutherland clay, clay-loam in 1990 and a Bradwell clay loam in 1991–1994. Seeding dates were 25, 7, 1, and 6 May in 1990, 1991, 1992, and 1994, respectively, for Experiment 1 and 5, 7, and 12 May in 1992, 1993, and 1994, respectively, for Experiment 2.

<sup>1</sup>NRCC No. 40025.

Publication no. C-1996-1004-05R. © 1996 National Research Council of Canada. For both experiments, fertilizer (11-51-0, N-P-K) was drilled in with the seed at a rate of  $\approx$ 50 kg/ha. Seeding rate was 250 seeds/m<sup>2</sup>. All field trials were established on fallow. Plots were harvested with a plot combine, and the grain was dried to a moisture content of 10 ± 1% in forced air driers at 30°C. Kernel weights were determined using a 500-kernel subsample, and test weight was measured using a 0.5 hl cup.

Total starch content was analyzed using the Megazyme total starch analysis (AA/AMG) procedure (McCleary et al 1994). The grain was milled in a Udy cyclone mill to pass through a 0.5-mm screen. A 100-mg sample was wet with 0.2 ml of ethanol and treated with thermostable  $\alpha$ -amylase (AA) to partially hydrolyze the starch. After completely dissolving the starch, dextrins were quantitatively hydrolyzed to glucose by amyloglucosidase (AMG). The amount of glucose was measured and the starch content was estimated as described by McCleary et al (1994).

Protein was measured in 300 mg of milled sample using the crude protein-Udy dye method (Method 46-14A, AACC 1983).

Analyses of variance (ANOVA) were performed for individual experiments and combined ANOVA was conducted. A random model was used. Repeatability for starch and protein content measurements were estimated from components of variance using the equation:

Repeatability = 
$$\sigma^2 g / (\sigma^2 g + \sigma^2 g \times y / n + \sigma^2 y / n r)$$

where  $\sigma^2 g = \text{component}$  of variance due to genetic differences among cultivars,  $\sigma^2 g x y = \text{variance}$  due to genotype × year interaction,  $\sigma^2 y = \text{variance}$  due to the environment, n = number of years, r = number of replicates. The term repeatability refers to the constancy of cultivar starch and protein concentration across years and replicates. Repeatability estimates and their standard errors were calculated according to Pesek and Baker (1971).

## **RESULTS AND DISCUSSION**

Significant ( $P \le 0.05$ ) cultivar variation for grain starch and protein concentration was detected in each of the seven field trials conducted. The average starch concentration was 68.6 and 69.8% for Experiments 1 and 2, respectively.

For Experiment 1, averaged over four years, the cultivars Fielder, Ford, and Potam had the highest starch concentration, while Ingal, Roblin, and PT516 had the lowest (Table I). For Experiment 2, averaged over three years, the Canada Prairie Spring wheat cultivars Biggar, Oslo, and Genesis produced the most starch, while the Canada Western Red Spring wheat cultivars AC Domain, Roblin, and Katepwa produced the least (Table I). For both experiments, the difference between the extreme cultivars was  $\approx 7\%$ . Lineback and Rasper (1988) in their review indicated that American wheats range from 63 to 72% in starch concentration, while European cultivars may be slightly higher. Thus, the

<sup>&</sup>lt;sup>2</sup>Crop Development Centre, 51 Campus Drive, University of Saskatchewan, SK, S7N 5A8, Canada.

<sup>&</sup>lt;sup>3</sup>Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9, Canada. Corresponding author. Fax 306/975 4839. E-mail: rchibbar@pbi.nrc.ca

 TABLE I

 Means for Grain Starch and Protein Concentration of Spring Wheat Cultivars Grown at Saskatoon, Canada

Experiment 1				Experiment 2			
Cultivar	Market Class <sup>a</sup>	Grain Starch, %	Grain Protein, %	Cultivar	Market Class <sup>a</sup>	Grain Starch, %	Grain Protein, %
Fielder	CSWS	71.6	12.9	Biggar	CPS	74.2	12.7
Ford	SWS	71.3	15.1	Oslo	CPS	72.9	14.2
Potam	SWS	69.9	14.5	Genesis	CPS	72.5	12.6
Genesis	CPS	69.8	12.6	Cutler	CPS	71.8	13.3
AUS1408	HWS	69.7	14.9	Columbus	CWRS	70.4	15.8
Kleiber	HRS	69.5	14.3	PT532	HRS	70.1	15.8
Park	CWRS	69.1	15.5	AC Minto	CWRS	69.8	15.4
Rongotea	HRS	68.7	14.4	Glenlea	CWES	69.8	13.5
Kenya 321	SWS	68.5	15.2	BW652	HRS	69.6	14.4
RL4137	CWRS	68.5	16.0	CDC Teal	CWRS	69.5	15.3
Katepwa	CWRS	68.4	15.8	CDC Makwa	CWRS	69.4	15.5
SUN56A	HWS	68.4	15.0	Laura	CWRS	69.0	15.0
Columbus	CWRS	68.1	16.7	Park	CWRS	68.9	15.0
AUS1293	SWS	67.9	15.1	Pasqua	CWRS	68.8	15.5
CDC Teal	CWRS	67.4	16.2	CDC Merlin	CWRS	68.3	15.0
Kenyon	CWRS	67.2	16.3	Katepwa	CWRS	68.1	15.4
Conway	CWRS	67.0	16.3	Roblin	CWRS	66.7	16.5
PT516	HRS	66.7	16.7	AC Domain	CWRS	66.6	16.6
Roblin	CWRS	65.7	16.8				
Ingal	HRS	64.6	17.4				
Pooled SE		0.7	0.4	Pooled SE		1.0	0.4
Year				Year			
1990		67.0	16.8	1990			
1991		69.4	15.4	1991			
1992		71.1	14.4	1992		67.8	14.9
1993				1993		71.5	14.9
1994		66.9	14.9	1994		70.0	14.8
Pooled SE		0.6	0.2	Pooled SE		0.8	ns

<sup>a</sup> CPS = Canada Prairie Spring, CWRS = Canada Western Red Spring, CWES = Canada Western Extra Strong, CWSWS = Canada Western Soft White Spring, HRS = Hard Red Spring, HWS = Hard White Spring, SWS = Soft White Spring.

<sup>b</sup> SE = standard error; ns = not significant.

 
 TABLE II

 Repeatability Estimates (± Standard Error) for Starch and Protein Concentration Measurements of Spring Wheat

	Replicates	Exper	iment 1	Experiment 2		
Years		Starch	Protein	Starch	Protein	
1	1	$0.52 \pm 0.10$	0.67 ± 0.11	$0.41 \pm 0.13$	0.71 ± 0.09	
	2	$0.63 \pm 0.10$	$0.70 \pm 0.11$	$0.48 \pm 0.15$	$0.77 \pm 0.08$	
2	1	$0.68 \pm 0.11$	$0.80 \pm 0.11$	$0.58 \pm 0.14$	$0.83 \pm 0.06$	
	2	$0.78 \pm 0.09$	$0.82 \pm 0.10$	$0.65 \pm 0.15$	$0.87 \pm 0.05$	
3	1	0.76 ± 0.09	$0.86 \pm 0.08$	0.67 ± 0.14	$0.88 \pm 0.05$	
	2	$0.84 \pm 0.09$	$0.88 \pm 0.10$	$0.73 \pm 0.14$	$0.91 \pm 0.04$	
4	1	$0.81 \pm 0.08$	$0.89 \pm 0.09$		• • •	
	2	$0.87 \pm 0.08$	$0.90 \pm 0.09$		•••	

range in starch concentration observed in the present study appears to be consistent with the limited published data.

In general, soft white spring wheat cultivars had higher starch concentration than did hard red spring cultivars (Table I, Experiment 1). In Experiment 2, Canada Prairie Spring (CPS) cultivars consistently had higher starch concentration than did hard red spring cultivars (Table I).

The difference in protein concentration for the extreme cultivars (Genesis and Ingal) was 4.5% in Experiment 1 and 4.0% (Genesis and AC Domain) in Experiment 2 (Table I).

Repeatability of starch content increased from 0.52 with a single determination to 0.87, based on four years of testing with two replicates per year (Table II). For Experiment 2, repeatability increased from 0.41 with a single determination to 0.73 with three years of testing (Table II). The repeatability for protein content (0.67–0.91) was higher than that for starch content, particularly with fewer replicates. Apparently, an additional one or two years of field testing would be required to obtain repeatabilities for starch content equivalent to those for protein. DeLaRoche and

Fowler (1975) reported a repeatability value of 0.98 for protein content determined using the Udy dye method. However, the repeatability value was based on multiple measurements of unreplicated grain samples. When environmental effects and genotype by environment interactions are taken into account, the repeatability estimates for protein content, as estimated by the Kjeldahl method, are lower (Baker and Campbell 1971)

In the current study, starch concentration and protein concentration were negatively correlated (Experiment 1, r = -0.82, P = 0.01; Experiment 2, r = -0.74, P = 0.01). Starch content tended to be associated with grain yield (r = 0.56, P = 0.01) for both experiments). The relationship between starch content and kernel or test weight was inconsistent. In Experiment 1, starch concentration and test weight were positively correlated (r = 0.52, P = 0.01) in 1992 and 1994 trials, but not in 1990 and 1991 trials (r = 0.02, ns). In Experiment 2, no correlation was observed between starch concentration and test weight (r = -0.01, ns).

### CONCLUSIONS

As might be expected the high yielding, low protein wheat types (CPS and SWS) had the highest starch concentration. Repeatability of starch concentration was generally lower than the repeatability for protein concentration. As is the case with all studies dealing with repeatability or heritability of a trait, the estimates are specific to genotypes and environments sampled.

### ACKNOWLEDGMENTS

The technical assistance of Ken Jackle and Cliff Mallard, and financial support from the Government of Saskatchewan and the National Research Council of Canada is gratefully acknowledged. We thank B. G. Rossnagel and R. T. Tyler, University of Saskatchewan, SK, for a critical review of the manuscript.

#### LITERATURE CITED

- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC, 8th ed. Method 46-14A, approved November 1972, revised October 1975 and September 1985. The Association: St. Paul, MN.
- BAKER, R. J., and CAMPBELL, A. B. 1971. Evaluation of screening tests for quality of bread wheat. Can. J. Plant Sci. 51:449-455.
- DE LA ROCHE, I. A., and FOWLER, D. B. 1975. Wheat quality evaluation. I. Accuracy and precision of prediction tests. Can. J Plant Sci. 55:241-249.
- HUCL, P. 1994. Repeatability of a simplified method for determining sprouting resistance in wheat. Plant Var. Seeds 7:79-84.
- LINEBACK, D. R., and RASPER, V. F. 1988. Wheat carbohydrates Pages 277-372 in: Wheat Chemistry and Technology. Vol. I. Y. Pomeranz. ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

- McLEARY, B. V., SOLAH, V., and GIBSON, T. S. 1994. Quantitative measurement of total starch in cereal flours and products. J. Cereal Sci. 20:51-58.
- MIURA, H., and TANII, S. 1994. Endosperm starch properties in several wheat cultivars preferred for Japanese noodles. Euphytica 72:171-175.
- NAKAMURA, T., YAMAMORI, M., HIRANO, H., HIDAKA, S., and NAGAMINE, T. 1995. Production of waxy (amylose-free) wheats. Mol. Gen. Genet. 248:253-259.
- PESEK, J., and BAKER, R. J. 1971. Comparison of predicted and observed responses to selection for yield in wheat. Can. J. Plant Sci. 51:187-192.
- WASSERMAN, B. P., HARN, C., MU-FORSTER, C., and HUANG, R. 1995. Progress towards genetically modified starches. Cereal Foods World 40:810-817.
- YAMAMORI, M., NAKAMURA, T., ENDO, T. R., and NAGAMINE, T. 1994. Waxy protein deficiency and chromosomal location of coding genes in common wheat. Theor. Appl. Genet. 89:179-184.

[Received May 5, 1996. Accepted August 23, 1996.]