Genotypic Effects on β-Glucan Content of Oat Lines Grown in Two Consecutive Years[1]

H. S. LIM,[2] P. J. WHITE,[2] and K. J. FREY[3]

ABSTRACT

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Oat lines (102 total) grown in 1989 and 1990 were analyzed for β-glucan by flow injection analysis (FIA) to determine the influence of variety and growing season on β-glucan contents. The correlation between FIA and the AACC enzymatic method for measuring β-glucan content was 0.97. The average coefficient of variation for the FIA measurements per sample was 1.2%, and the oat lines showed a normal distribution for β-glucan contents in both years, ranging from 3.0 to 6.5% in 1989 and from 3.9 to 6.4% in 1990. Mean β-glucan contents for the two years were the same, 5.1%, but oat varieties differed significantly, even when tested via the mean square for year × variety.

Oat (*Avena sativa* L.) is an ingredient in many breakfast cereals consumed in the United States. Oat, as well as barley (*Hordeum vulgare* L.), contains (1→3),(1→4)-β-D-glucan (β-glucan), which is a water-soluble dietary fiber with possible therapeutic value and use as a food hydrocolloid with desirable rheological properties (Anderson et al 1984, Wood and Weisz 1984, Klopfenstein 1988). On the other hand, β-glucan in barley affects malting and brewing adversely because of its water-absorbing properties. Also, poultry fed a high β-glucan ration show depressed weight gain because this component has low metabolizable energy (Anderson et al 1978, Bamforth 1982, Newman et al 1987, Jørgensen 1988). For these reasons, producing oat lines with either high or low contents of β-glucan, depending on their use, is of interest.

Several enzymatic methods give simple, fast, and reliable quantification of β-glucans (Ahluwalia and Ellis 1984, Henry 1984, McCleary and Glennie-Holmes 1985). But an interaction of some dyes with cereal β-glucans (Wood and Fulcher 1978) has caused researchers to develop a nonenzymatic method of analysis for β-glucan. Recently, an automated flow injection analysis (FIA) method, based on the measurement of intensity of fluorescent absorption of a dye-β-glucan complex, was developed for rapidly quantifying β-glucan content (Jørgensen 1988, Jørgensen and Aastrup 1988). Slight modifications of this FIA method were introduced by Paisley and Zygmunt (1989).

The objectives of this study were 1) to determine the range, effect of year, year × variety interaction, frequency distribution, and mean of β-glucan contents in various oat lines, and 2) to estimate the variability for β-glucan content measured by FIA within an oat variety.

MATERIALS AND METHODS

A set of 102 oat lines, including old-time and current varieties, experimental lines from the U.S. north central region, and accessions of *A. sterilis* (the progenitor of cultivated oats), were grown in a two-replicate experiment at Ames, IA, in 1989 and 1990. A plot was hill-sown with 30 seeds, and hills were spaced 1 m apart in perpendicular directions. The experiment included two check varieties, Hamilton and Noble, at regular intervals in the array of accessions. The oat varieties and experimental lines represented a broad range of genotypes adapted in the north central region. The *A. sterilis* accessions were collected from various countries surrounding the Mediterranean Sea.

The 1989 growing season for oat, although drier than normal, produced good yields of plump oat groats (caryopses). The 1990 growing year had normal weather conditions. The weight of seeds from the plots varied, but generally each produced at least 10 g of groats. The groat samples were stored at 4°C and 45% relative humidity until they were ground for analyses. A total of 408 oat samples and 96 oat reference samples were analyzed by the FIA method. Oat reference samples and purified oat β-glucan for standards were provided by the Quaker Oats Company (Barrington, IL).

Procedures

Two methods were used to measure the β-glucan contents in oat. All samples were analyzed via the automated FIA procedure developed by Jørgensen and Aastrup (1988) and slightly modified by Paisley and Zygmunt (1989) to improve the accuracy of β-glucan measurements through calibration of the standard and instrument together with a correction factor. Because this FIA modification has not yet appeared in print, the entire procedure is described below. The FIA equipment (Quick Chem, Lachat Instruments, Milwaukee, WI) consisted of a random access sampler, proportional pump, injection module, fluorescence spectrophotometer (F-1050, Hitachi, Tokyo, Japan), monitor, computer, and printer.

The β-glucan contents of oat reference samples also were analyzed by using the AACC enzymatic method (AACC 1983), which was updated by Zygmunt and Paisley (in press). These data were the mean of triplicates, and values were provided by the Quaker Oats Company or obtained from analyses in our laboratory.

FIA Sample Preparation

About 10 g of a sample was dried at 70°C for 18 hr to inactivate the endogenous β-glucanase and to dry the groats, and the dried sample was ground on an ultracentrifugal mill (ZM-1, Retsch GmbH & Co., Haan, Germany) with a 0.5-mm screen. Dry basis sample weights were obtained via duplicate moisture determinations of ground sample powder (2 g) at 130°C for 2 hr in a convection oven. To extract the soluble β-glucan, about 100 mg of oat flour from a sample was weighed and placed into a 20- × 150-mm screw cap culture tube with 4.9 ml of water and 0.1 ml of 0.2% thermostable α-amylase (*Bacillus subtilis,* Calbiochem, La Jolla, CA) solution. A magnetic stirring vane was placed into the tube, and the tube was sealed, heated with stirring in a 100°C water bath for 1 hr, and cooled in a 13–15°C water bath for 10 min. Then, 5.0 ml of 0.075*M* H_2SO_4 was added to the tube, and the tube was reheated for exactly 10 min in a 100°C water bath with stirring and then cooled for 10 min in a 13–15°C water bath. Next, an aliquot of the sample was transferred to a 2-ml polypropylene tube and centrifuged for 11 min at 10,000 × *g* (Microfuge 12, Beckman, Palo Alto, CA). After centrifugation, three 0.5-ml aliquots of the supernatant from the sample were measured into three 12- × 75-mm culture tubes with an automatic diluter (Micro Lab 1000, Hamilton, Bonaduz,

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[2]Postdoctoral research associate and associate professor, respectively, Department of Food Science and Human Nutrition and Center for Crops Utilization Research, 3367 Dairy Industry Building, Iowa State University, Ames 50011.

[3]Distinguished professor, Department of Agronomy, Iowa State University, Ames 50011.

Switzerland). Each of these tubes was diluted to 3 ml with distilled water before measurement by FIA.

FIA

The tubes with the diluted samples were loaded on the random access sampler and measured automatically according to the controller program by passing through the pump, injection module, and fluorescence spectrophotometer. Each tube was measured three times for β-glucan content. Therefore, the β-glucan value for a sample of oat groats was the mean of nine measurements (i.e., three tubes × three measurements per tube). A 0.1 *M* Tris solution (Trizma 8.0 preset crystals, Sigma, St. Louis, MO) and a 0.005% (w/v) cellufluor (Calcofluor, Polysciences Inc., Warrington, PA) solution were filtered through a 0.45-μm nylon membrane filter and used as sample carrier and fluorescence color reagent, respectively, for FIA. The diluted oat sample and the buffer and reagent solutions were kept free of lint and dust that might clog tubings and valve connections in the FIA apparatus, and each valve was regularly cleaned for better flow. Sample injection volume was controlled by time at pump speed 35. Sampler/valve loading time and injection time were 10 and 15 sec, respectively, with a 30-sec cycle period.

Data Validity and Reproducibility

Six concentrations (250, 200, 150, 100, 50, and 30 mg/L) of purified (93% pure) oat β-glucan solution were used as calibration standards. A correlation coefficient of ≥ 0.995 was required for a run of calibration standards to be accepted. A standard solution of β-glucan (150 mg/L) was used in every 13th sample tube as a check on the procedure. Oat bran and an oat reference sample, each with known β-glucan content, were included in each FIA tray of tubes (96 tube cells per tray) to check the validity of the tray. If the β-glucan contents of the known samples deviated from the known values by ≥ 3 sec (standard deviation), the results from that sample tray were rejected.

Calculation

The computer was programmed to determine the maximum increase in fluorescence intensity (peak area) of the sample and to convert the measured peak area to milligrams of β-glucan per liter according to the regression line constructed from the tubes with purified oat β-glucan solution. β-Glucan in milligrams per liter was converted to percent β-glucan (dry basis) according to the following equation

$$\text{Percent } \beta\text{-glucan} = R/W \times \text{DF} \times 0.01 \text{ L} \times 100,$$

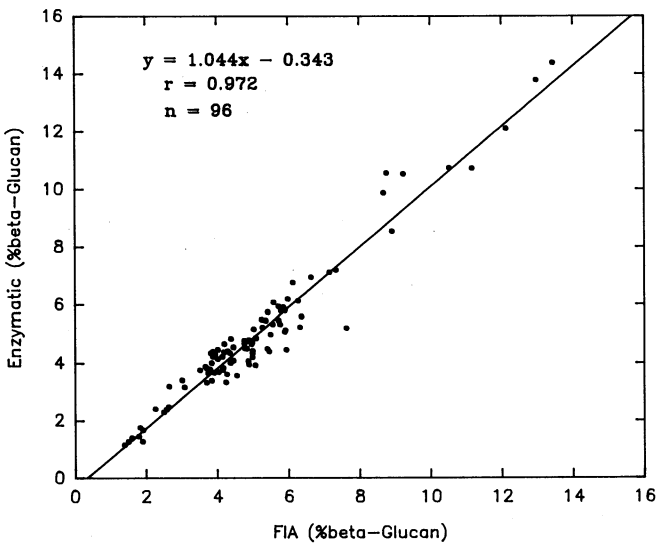

Fig. 1. Coincidence, regression, and correlation of β-glucan contents of oat groats and oat bran measured by the flow injection analysis (FIA) and enzymatic procedures.

where R = β-glucan concentration determined by FIA in milligrams per liter, W = sample weight in milligrams (dry basis), DF = dilution factor (3 ml/0.5 ml = 6), and 0.01 L = final volume of extraction solution (10 ml).

Statistical Analysis

An analysis of variance and paired-comparison t tests were used to determine whether differences existed either among oat varieties or between growing years or replications. The univariate procedure of SAS (SAS Institute Inc., Cary, NC) was used to plot the distribution of β-glucan contents of 102 oat lines grown each year into bar charts.

RESULTS AND DISCUSSION

Validity of FIA Method

Ninety-six oat reference samples were analyzed by both enzymatic and FIA methods. Because the enzymatic method is widely accepted for quantification of β-glucan, the relation between the β-glucan contents in oat determined by the enzymatic method (ENZ) and the FIA method is shown in Figure 1. The linear correlation coefficient was 0.97 with the following linear regression:

$$\text{Percent } \beta\text{-glucan (ENZ)} = 1.044 \times \text{percent } \beta\text{-glucan (FIA)} - 0.343.$$

This high correlation reaffirms the accuracy of FIA for quantification of β-glucan. The FIA results were converted to percent β-glucan as measured by the enzymatic procedure via the regression equations for each sample. By applying the above correction equation via this modified FIA method, it was possible

TABLE I
Contents of β-Glucan Measured by Flow Injection Analysis in 31 Random Oat Lines Grown in 1990

	β-Glucan (%, db[a])					
Line	Rep[b] 1	CV[c]	Rep 2	CV	Average	Rep 1:Rep 2[d]
Andrew	6.1	0.8	5.5	0.7	5.8	1.11
D826-922	5.7	1.5	5.4	0.7	5.6	1.06
MO07929	6.1	0.7	4.9	0.5	5.5	1.24
Starter	5.3	1.0	5.8	1.5	5.5	0.91
H730-12	5.6	0.8	5.3	1.4	5.5	1.06
Bates	5.5	1.5	5.5	2.4	5.5	1.00
IL82-2154	5.6	1.3	4.6	1.3	5.4	1.22
PA8598-11662	5.8	1.2	5.1	0.9	5.4	1.14
D921-643	5.0	0.3	5.7	2.3	5.4	0.89
Porter	4.8	1.0	5.9	0.2	5.4	0.81
H689-8	5.7	1.2	5.0	0.6	5.4	1.14
Don	5.5	0.4	4.8	0.2	5.3	1.15
Hazel	5.0	0.4	5.6	1.4	5.3	0.89
Steele	4.7	1.2	5.9	2.0	5.3	0.80
Otee	5.5	0.5	4.9	0.2	5.2	1.12
H688-4	4.9	1.4	5.1	2.3	5.2	0.96
Y849-9-2	5.4	0.9	4.6	1.8	5.1	1.17
Larry	4.8	0.5	5.1	1.8	5.0	0.94
Noble	5.5	0.2	5.2	1.8	5.0	1.06
PA8494-4099	5.0	1.4	4.7	1.1	4.9	1.06
IL85-6264-1	4.9	0.7	4.8	2.0	4.9	1.02
H19-12	4.4	0.9	5.4	2.0	4.9	0.81
H696-8	4.0	0.4	5.4	2.8	4.9	0.74
H689-13	4.3	0.7	4.0	0.8	4.6	1.08
MN87194	4.6	1.0	4.4	1.7	4.5	1.05
OH1014	4.6	0.7	4.2	2.3	4.4	1.10
WIX5229-1	4.2	0.9	4.4	0.8	4.3	0.95
H19-14	4.5	2.3	4.1	0.7	4.3	1.10
Montezuma	4.4	1.9	4.1	1.9	4.3	1.07
H28-5	4.4	1.3	4.0	1.2	4.2	1.10
MN86109	4.0	1.5	4.1	0.0	4.1	0.98

[a] Dry basis.
[b] Replication.
[c] Coefficient of variation.
[d] Overall mean = 1.024, $S_x = 0.129$, $n = 31$.

to calibrate the standard and the instrument together (Paisley and Zygmunt 1989).

Variability of FIA Results

The coefficients of variation (CV) for the nine measurements (three aliquots [tubes] × three extracts [measurements]) of β-glucan content on an entry-plot basis for 31 random oat lines grown in 1990 ranged from 0 to 2.8% with an average value of 1.2% (Table I). The batch-by-batch or day-by-day CV for oat bran as a control was 3.0% for all 25 analyses done in a period of two months. This CV value gives an estimate of all variability of the sample preparation and methodology. Wood et al (1991) reported an average CV of 3.6% attributable to analytical replication of the enzymatic method for β-glucan analysis. To minimize variations due to sample and batch-to-batch preparations, two known β-glucan samples were included in every batch of samples starting from the drying step. The paired ratios of replication 1 to replication 2 listed in Table I have a mean value of 1.024 and a standard deviation of 0.127. These ratios show the combined variability of error from the FIA procedure (including sample preparation, batch-to-batch extraction, and FIA measurement) and from replication of planting site (replication 1 vs. replication 2).

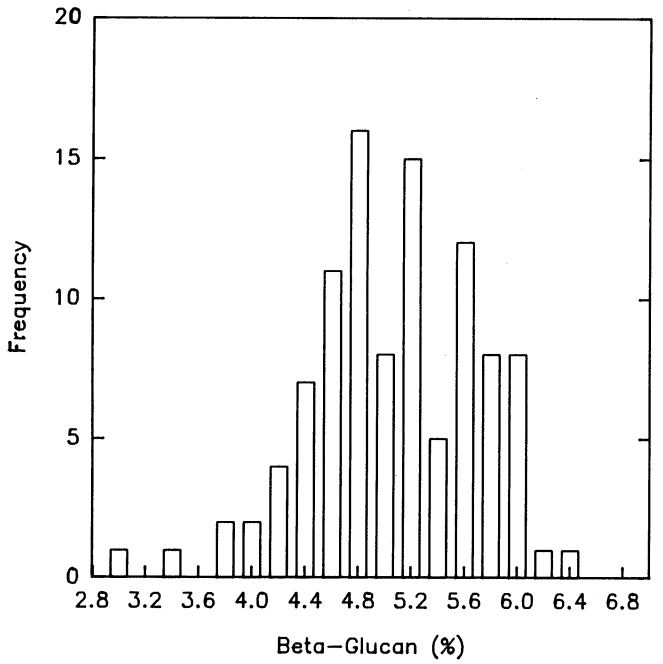

Fig. 2. Frequency distribution for β-glucan contents of 102 oat lines grown in 1989.

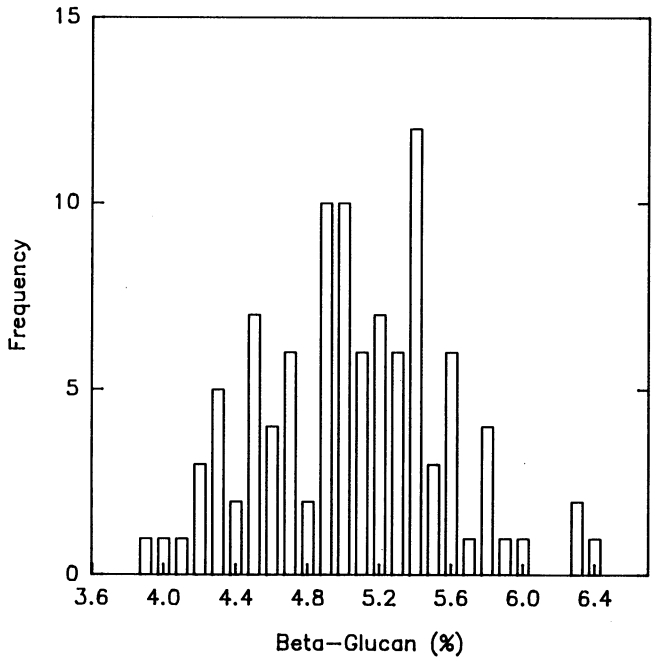

Fig. 3. Frequency distribution for β-glucan contents of 102 oat lines grown in 1990.

Analyses of Oat Samples

The frequency distribution for β-glucan contents for the 102 oat lines grown in 1989 was normal, with mean and mode equal to 5.10 and 4.8, respectively (Fig. 2). The interquartile range (25–75%) of β-glucan contents for 1989 samples was 4.7–5.6, and maximum and minimum values were 6.5 and 3.0%, respectively. In 1990, the frequency distribution for the same lines was normal, with mean and mode equal to 5.05 and 5.4, respectively (Fig. 3). The 1990 interquartile range was 4.7–5.4, and maximum and minimum values were 6.4 and 3.9%, respectively. The means for β-glucan content (5.10 for 1989, 5.05 for 1990) and the ranges (3.0–6.5 for 1989, 3.9–6.4 for 1990) for the 102 different oat lines

TABLE II
Mean Squares from an Analysis of Variance of the β-Glucan Contents of Oat Grown in 1989 and 1990

Source	Degrees of Freedom	Mean Squares	*F* Value
Year	1	0.181	1.71
Replicate (year)	2	0.153	1.48
Varieties	101	0.938	8.84***[a]
Year × varieties	101	0.399	3.76***
Error within years	202	0.106	

[a]***, $P < 0.0001$.

TABLE III
Content of β-Glucan[a] and Rank for 15 Highest and 15 Lowest Oat Lines When Averaged Over 1989 and 1990

	1989		1990		Average	
Line	β-Glucan (%)	Rank	β-Glucan (%)	Rank	β-Glucan (%)	Rank
Highest in β-glucan						
IL85-6183-1	6.0	5	6.3	2	6.1	1
MN86226	6.1	3	5.9	4	6.0	2
MO07929	6.5	1	5.5	12	6.0	2
ND84769	6.0	5	5.8	5	5.9	4
H87-7-4	5.9	9	5.8	5	5.9	4
Starter	6.3	2	5.4	14	5.8	6
D831-1-571	5.9	9	5.7	8	5.8	6
H730-12	6.1	3	5.5	12	5.8	6
D921-255	6.0	5	5.6	9	5.8	6
Andrew	5.7	11	5.8	5	5.7	10
Proat	4.9	15	6.4	1	5.7	10
Bates	6.0	5	5.3	15	5.6	12
OH1011	5.7	11	5.6	9	5.6	12
Clinton	5.6	13	5.6	9	5.6	12
Gopher	5.2	14	6.0	3	5.6	12
Lowest in β-glucan						
H696-8	4.2	93	4.9	61	4.6	87
PA8393-11138	4.6	92	4.5	91	4.6	87
H713-29	4.0	98	5.1	88	4.5	89
H19-14	4.7	91	4.3	96	4.5	89
SD85009	4.8	89	4.1	100	4.5	89
H553	4.2	95	4.6	89	4.4	92
OH1014	4.6	92	4.2	98	4.4	92
H45-8	4.8	89	3.9	102	4.3	94
Storemont	4.2	95	4.5	91	4.3	94
Victorgrain	4.2	95	4.5	91	4.3	94
H28-5	4.4	94	4.2	98	4.3	94
MN86108	3.9	99	4.5	91	4.2	98
Kota	4.1	97	4.3	96	4.2	98
Montezuma	3.5	101	4.4	95	4.0	100
ND840341	3.8	100	4.0	101	3.9	101
H23-2	3.0	102	4.6	89	3.8	102

[a]Dry basis.

TABLE IV
Average β-Glucan Content Measured by Flow Injection Analysis in 102 Oat Lines[a] Grown in 1989 and 1990

Line	Average Percent β-Glucan	Line	Average Percent β-Glucan
IL82-2154	5.6	H79-2	5.0
Jaycee	5.6	Larry	5.0
PA8598-11662	5.5	Pacer	5.0
D826-356	5.5	PA8598-8415	5.0
P7869D1-5-3-2-10-1	5.5	H688-4	5.0
Dal	5.5	MO08139	5.0
Don	5.5	MN87187	5.0
H688-11	5.5	MO07941	5.0
WIX5445-4	5.5	MO08054	5.0
Hazel	5.5	Ogle	5.0
H702-4	5.5	Y949-9-2	5.0
Ensiler	5.4	SD840104	4.9
D826-922	5.5	Hamilton	4.9
Marion-Canadian	5.3	Noble	4.9
Otee	5.3	D920-51	4.9
D831-1-308	5.3	Horicon	4.9
D921-643	5.3	H19-12	4.9
IL84-3093	5.3	OH1012	4.9
MO08291	5.3	H689-8	4.9
PA8494-4099	5.3	MN84231	4.8
H722-23	5.2	H27-5	4.8
OH1006	5.2	MN86109	4.8
Steele	5.2	PA85494-11717	4.8
H52-5	5.2	PA8393-15050	4.8
Porter	5.2	IL83-8037-1	4.7
P7869D1-5-17-3	5.2	MN87194	4.7
Multiline-E77	5.2	Richland	4.7
PA8598-4200	5.2	Clintford	4.7
H73-9	5.2	Webster	4.7
IL85-6264-1	5.1	H61-3-3	4.7
Hytest	5.1	H695-1	4.7
PA8393-1500	5.1	H722-9	4.7
Valley	5.1	MN87189	4.7
Y933-11-2	5.1	Burnett	4.7
WIX5229-1	5.1	H689-13	4.6
H75-117	5.0	H6996-8	4.6

[a]The 15 highest and 15 lowest oat lines in β-glucan content are listed in Table III.

were similar to the mean (5.9) and range (3.9–6.8) found for 11 oat varieties (Wood et al 1991). Iowa weather in 1989 was drier and hotter than that in 1990, but the β-glucan means for the two years were not different (Table II). Highly significant differences ($P < 0.0001$) occurred among the oat lines for β-glucan content. These results suggest that the β-glucan content of oat is an inherited trait and, as such, oat varieties with high or low β-glucan content could be developed. Other studies (Peterson 1991, Welch et al 1991) also have shown strong genotypic effects of oat cultivars on β-glucan content. As shown in Table III, the rankings of oat lines on the basis of β-glucan content were similar in 1989 and 1990, at least for the highest and lowest lines. But, as shown by the analysis of variance (Table II), a significant interaction occurred for years and oat lines. Our data suggest that even though the interaction was significant, it would not be of much practical importance in a variety development program (Peterson 1991). The β-glucan contents of lines not shown in Table III, averaged over 1989 and 1990, are listed by rank in Table IV.

In summary, the FIA procedure gave consistent sample readings with an average CV of 1.2% within a sample measurement and an average CV of 3.0% for oat bran among batches and days. Means of β-glucan contents for the 102 oat lines grown in 1989 and in 1990 were not different. Further, the mean square for varieties was significantly greater than that for year × variety interaction. Our results demonstrated that the β-glucan content of oat is highly predictable, at least for oat lines at the tails of the frequency distribution.

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