Mixed Linkage β-Glucan, Protein Content, and Kernel Weight in *Avena* Species[1]

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ABSTRACT

Eighteen species of *Avena,* including nine diploids, four tetraploids, and five hexaploids, were grown in one location and assayed for mixed-linkage (1→3),(1→4)-β-D-glucan (β-glucan) content. Five domestic cultivars of *A. sativa,* representing the range of β-glucan currently available in Canadian oats, were grown at the same time for comparison. The range of β-glucan content in the samples assayed was 1.8–5.5%, with significant differences observed between species and also between groups based on ploidy. Molar ratios of 3-*o*-β-cellobiosyl-D-glucose to 3-*o*-β-cellotriosyl-D-glucose released by lichenase digestion of the β-glucan were also determined. This ratio provides an indicator of the major structural features of the β-glucan. Although some differences were apparent between species, there was little overall variation, indicating that β-glucan structure is generally a conserved feature in these samples. Protein content and 1,000-kernel weight were also determined for all samples. A negative relationship was observed between protein content and 1,000-kernel weight: the non-*sativa* species, in general, had small seeds and high protein content; the domestic cultivars had large seeds and relatively low protein content. A weak negative relationship was also observed between β-glucan and protein content. No relationship was apparent between β-glucan content and 1,000-kernel weight.

(1→3),(1→4)-β-D-Glucans (β-glucans) are cell-wall polysaccharides found almost exclusively in the grasses (Stinard and Nevins 1980). The highest levels of β-glucans are found in the grains of oats and barley (Fincher and Stone 1986). The major repeating subunits of cereal β-glucans are β-(1→4)-linked trisaccharides and tetrasaccharides (3-*o*-β-cellobiosyl-D-glucose and 3-*o*-β-cellotriosyl-D-glucose, respectively) that are released by lichenase digestion of the polysaccharide. These oligosaccharides make up approximately 85–90% of the total β-glucan of oats (Wood et al 1991). Differences in the ratio of trisaccharides to tetrasaccharides reveal structural differences in β-glucans that are not apparent from analysis of the percentage of (1→3) and (1→4) linkages (Wood et al 1991). Structural differences might influence physical properties and, hence, physiological response.

Although, traditionally, the largest use for oats has been as animal feed, the potential for oat products to reduce serum cholesterol (Anderson et al 1990) has generated an increased demand for oats in human nutrition. The influence of oats and oat products on serum cholesterol has been attributed to the high β-glucan content of the grain (Anderson et al 1990). In animal nutrition, too much β-glucan in the diet may be detrimental; lower and slower weight gains have been reported in young chicks fed diets supplemented with β-glucan (Cave et al 1990).

The increasing demand for oats containing high or low levels of β-glucan (for human or animal diets) generates a requirement to broaden the range available to better serve specific markets. A limited screening of wild *Avena* species revealed a wider range of β-glucan content and a higher protein content than that observed in domestic oat cultivars (Welch et al 1991). A more comprehensive screening of non-*sativa* germ plasm increases the possibility of finding genotypes with higher or lower levels of β-glucan than are currently available. These genotypes might then be used in a breeding program to develop oat cultivars for specific markets.

Environmental influences affect β-glucan content in oats (Peterson 1991, Lim et al 1992, Miller et al, *in press*). In the present investigation, 18 species of *Avena* and five cultivars of domestic *A. sativa,* covering the range of β-glucan content currently reported in Canadian oats (Miller et al, *in press*), were grown in the same season at the same location to minimize environmental effects. β-Glucan content, molar ratios of trisaccharides to tetrasaccharides, protein content, and 1,000-kernel weight were determined for all samples.

MATERIALS AND METHODS

Samples

Accessions of nondomestic oats were obtained from Plant Gene Resources, Agriculture Canada. Domestic cultivars and the breeder's line 0A516-2 were the generous gift of V. D. Burrows, Plant Research Centre, Agriculture Canada. Eighteen species of *Avena,* including nine diploids, four tetraploids, five hexaploids, and five cultivars of *A. sativa* were germinated in the dark on moist filter paper and transplanted to pots when growth began. When the seedlings were approximately 2–5 cm high, the pots were moved into a growth chamber for vernalization (16-hr light at 2°C, 8-hr dark at 0°C) for four weeks before transplanting in the field. Plants were grown in random plots, in the same field, in the 1988 growing season. Seeds were harvested as they matured to prevent loss and cross-contamination due to shattering. For β-glucan analysis, samples were dehulled manually. The resulting groats were assayed with a commercial kit (Biocon β-Glucan Assay Kit, Biocon, Lexington, KY) using a modification (Miller 1992) of the McCleary enzymatic method (McCleary and Glennie-Holmes 1985). The method of Wood et al (1991), modified for high-performance anion-exchange chromatography with pulsed-amperometric detection, was used to determine trisaccharide-to-tetrasaccharide ratios. All determinations of β-glucan and trisaccharide-to-tetrasaccharide ratios were performed in triplicate. The 1,000-kernel weights were calculated from the weight of 150 kernels. Nitrogen determinations were performed in duplicate using a Kjeltec Auto 1030 Analyzer (Tecator, Höganäs, Sweden). Protein was estimated as N × 6.25. Statistical analyses were performed using the SAS System (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

The β-glucan content, molar ratios of trisaccharides and tetrasaccharides, protein content, and 1,000-kernel weight of the 18 nondomestic species of *Avena* and five domestic cultivars of *A. sativa* are presented in Table I. The domestic cultivars represent the range of β-glucan content observed for Canadian cultivars (Miller et al, *in press*). β-Glucan content in the non-*sativa* species ranged between 1.8 and 5.5%, with significant differences between species (Duncan's multiple range test, $\alpha = 0.05$). The lower limit of this range is well below the low level of the β-glucan domestic cultivars grown in the same experiment for comparison (Tibor, 3.7% and 0A516-2, 3.8%). The upper limit of β-glucan content in the 18 nondomestic species studied (*A. hirtula,* 5.5%), although significantly higher, was much closer to that in the domestic cultivars with the highest β-glucan content reported to date (Marion, 5.0% in this growing season and location). The domestic cultivars separated into groupings similar to those already observed for Canadian oats (Miller et al, *in press*).

The diploids contained the species with both the lowest (*A.*

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eriantha, 1.8%) and the highest (*A. hirtula,* 5.5%) levels of β-glucan. This group also had the greatest number of species (nine). The tetraploids, a much smaller group (only four species), had a much narrower β-glucan range: 2.4% (*A. magna*) to 3.9% (*A. vaviloviana*). The non-*sativa* hexaploids (five species) also had a narrower β-glucan range than the diploids: 4.3% (*A. occidentalis*) to 5.3% (*A. hybrida*). The domestic hexaploids (five cultivars), which were considered separately because they all belong to the same species (*A. sativa*), had a wider range of β-glucan than the nondomestic: 3.7% (Tibor) to 5.0% (Marion). Within the non-*sativa* groups, the β-glucan contents of most of the species were significantly different from each other.

In addition to differences between species, a comparison of the means for each group (Duncan's multiple range test, $\alpha = 0.05$) showed significant differences in β-glucan content with respect to ploidy level in the samples studied. The tetraploids, as a group, had a significantly lower mean β-glucan content (3.2%) than the nondomestic hexaploids (4.9%). The mean β-glucan content for the five domestic cultivars (4.2%) was not significantly different from that of the nondomestic hexaploids. The mean β-glucan content of the diploids was 3.5%, which is not significantly different from the means for either the tetraploids or the domestic hexaploids. The mean β-glucan content for the nondomestic hexaploids was significantly higher than for the diploids.

The range of β-glucan contents observed in the present study was similar to the 1.2–5.7% range reported in a study of eight greenhouse-grown nondomestic species (Welch et al 1991), which included four diploids, three tetraploids, and one hexaploid. Although the absolute values for β-glucan content for the eight species reported by Welch et al (1991) were different for the same species in the present study, the ranking of the species was similar for the two studies. However, no relationship was found between ploidy level and kernel β-glucan by Welch et al (1991).

The range of trisaccharide-to-tetrasaccharide molar ratios observed in all of the samples measured was 1.81–2.33. Duncan's multiple range test ($\alpha = 0.05$) places these samples into 10 groups. Although many of these groups show considerable overlap, statistically significant differences in molar ratios of trisaccharides to tetrasaccharides can be seen between some species. The range of molar ratios in the domestic cultivars alone was 2.05–2.11, but although the ratios at the two extremes are significantly different, in general, the intermediate ratios are not. These values are in close agreement with previous studies of domestic cultivars (Wood et al 1991, Miller 1992). It seems unlikely that such subtle structural differences would significantly influence the physical characteristics of the polysaccharide (Buliga et al 1986).

Structural differences in β-glucan in grains, as indicated by the trisaccharide-to-tetrasaccharide ratios, have been reported between different genera of the cereals. Values are highest in wheat (3.04–3.84) and barley (2.93–3.41); one rye sample is intermediate (2.73; Wood et al 1991). The results reported here suggest that there are some differences within genera as well, although β-glucan structure, overall, appears to be a generally conserved feature in these *Avena* samples.

Protein levels varied between species, and, in general, were much higher in the nondomestic species than in the domestic cultivars. Mean protein levels in the diploids (29.1%) and tetraploids (27.4%) were not significantly different from each other, but they were significantly higher than the domestics (20.7%, Duncan's multiple range test, $\alpha = 0.05$). The protein content observed for nondomestic oats in the present study was similar to that reported by Welch et al (1991) for eight nondomestic species, although the ranking of the species was slightly different in the two studies.

The domestic hexaploids, with a mean 1,000-kernel weight of 31.9 g, showed a significant yield advantage over all of the non-

TABLE I
β-Glucan Content, Molar Ratio of Trisaccharide and Tetrasaccharide, Protein Content, and 1,000-Kernel Weight in *Avena* Groats[a]

Avena Samples	β-Glucan[b]	Molar Ratio	Protein, %[c]	1,000-Kernel Weight, g
Diploids				
A. eriantha	1.8 ± 0.10 l	2.11 ± 0.04 c–g	30.7 d	4.7
A. ventricosa	2.3 ± 0.7 k	2.12 ± 0.08 c–g	33.7 a	5.0
A. canariensis	2.8 ± 0.04 j	2.33 ± 0.05 a	23.3 k	11.4
A. pilosa	2.9 ± 0.03 ij	1.88 ± 0.07 ij	33.1 b	4.0
A. longiglumis	3.1 ± 0.07 gh	2.33 ± 0.05 a	31.3 c	9.0
A. clauda	3.2 ± 0.01 g	2.10 ± 0.02 c–g	33.0 b	3.5
A. strigosa	4.6 ± 0.03 d	2.15 ± 0.02 b–f	20.0 p	18.5
A. lusitanica	5.1 ± 0.07 bc	2.33 ± 0.19 a	26.9 h	8.7
A. hirtula	5.5 ± 0.09 a	2.24 ± 0.03 ab	30.2 e	3.3
Mean	3.5 B	2.18 A	29.1 A	7.6 C
Tetraploids				
A. magna	2.4 ± 0.18 k	1.96 ± 0.12 hi	29.8 f	32.1
A. murphyi	3.0 ± 0.04 hi	1.81 ± 0.06 j	28.2 g	30.7
A. barbata	3.7 ± 0.13 f	2.19 ± 0.05 b–e	28.4 g	5.7
A. vaviloviana	3.9 ± 0.07 f	2.11 ± 0.02 c–g	23.0 k	9.4
Mean	3.2 B	2.02 A	27.4 AB	19.5 B
Hexaploids				
A. occidentalis	4.3 ± 0.08 e	2.16 ± 0.04 b–f	24.2 ij	11.9
A. fatua	4.5 ± 0.03 d	2.01 ± 0.02 gh	22.6 l	17.9
A. byzantina	5.0 ± 0.10 c	2.12 ± 0.04 c–g	21.6 n	24.4
A. sterilis	5.2 ± 0.13 bc	2.14 ± 0.06 b–f	24.4 i	12.6
A. hybrida	5.3 ± 0.13 b	2.09 ± 0.04 d–g	23.9 j	11.5
Mean	4.9 A	2.10 A	23.3 BC	15.7 BC
Domestic cultivars of *A. sativa*				
Tibor	3.7 ± 0.16 f	2.11 ± 0.01 c–g	21.9 mn	31.7
OA516-2	3.8 ± 0.11 f	2.10 ± 0.05 c–g	21.7 mn	31.5
Woodstock	4.1 ± 0.05 e	2.06 ± 0.05 f–h	22.1 m	28.9
Donald	4.2 ± 0.10 e	2.11 ± 0.06 c–g	16.7 q	31.5
Marion	5.0 ± 0.12 c	2.04 ± 0.04 f–h	21.2 o	36.1
Mean	4.2 AB	2.08 A	20.7 C	31.9 A

[a]Values and means in each column with the same lowercase or same uppercase letter are not significantly different (by Duncan's multiple range test, $\alpha = 0.05$).
[b]Mixed linkage (%, dry weight basis).
[c]Dry weight basis.

cultivated groups. Welch et al (1991) also observed lower kernel weights for nondomestic species of *Avena* than for domestic cultivars.

The high protein content and wider range of β-glucan content observed in the nondomestic oats are useful qualities for inclusion in a breeding program. With the exception of *A. magna* and *A. murphyi,* however, these attributes are accompanied by much smaller seed sizes than commercial cultivars (Pearson correlation coefficient $r = -0.63$ for all of the species and cultivars). The relationship between β-glucan and protein content, although weaker, was also negative ($r = -0.54$). There was no apparent relationship between β-glucan content and 1,000-kernel weight ($r = 0.13$). In addition, interspecific crossing is a difficult and time-consuming process. In light of these considerations, the possibilities for extending the high and low limits of β-glucan content in domestic oats by interspecific crossings with some of the non-*sativa* species may be limited.

Although the range of β-glucan contents observed in the nondomestic species was wider than the range seen in the domestic cultivars, the range extension was restricted to the lower end. In barley, low β-glucan content is a dominant characteristic (Greenberg 1977), although additive effects were also significant (Lance 1984). If β-glucan inheritance is similar in oats, breeding a lower or higher β-glucan oat could be more efficiently achieved by appropriate crosses using hexaploid material from domestic *A. sativa.* The use of mutagens to produce low β-glucan mutants in barley has also been reported (Aastrup 1983) and may find application in oats as well.

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