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Selection for Greater β-Glucan Content in Oat Grain

C. T. Cervantes-Martinez
Universidad Autonoma Chapingo

Kenneth J. Frey
Iowa State University

Pamela J. White
Iowa State University, pjwhite@iastate.edu

D. M. Wesenberg
United States Department of Agriculture

J. B. Holland
United States Department of Agriculture

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C. T. Cervantes-Martinez, K. J. Frey, P. J. White, D. M. Wesenberg, and J. B. Holland*

ABSTRACT

Oat (*Avena sativa* L.) β-glucan lowers serum cholesterol in humans. Development of oat cultivars with greater groat (caryopsis) β-glucan content would increase the nutritional and economic value of the crop. The objectives of this experiment were to evaluate the response to phenotypic selection among individual S_0 plants for greater groat β-glucan content in two genetically broad-based populations; to compare selected experimental lines to standard check cultivars; and to estimate genetic variances and heritabilities and to test for nonadditive genetic variance for β-glucan content. We measured groat β-glucan contents of check cultivars and parental lines and random $S_{0:1}$ lines from initial and selected generations of each population grown in field experiments in 1996 and 1997 at two Iowa locations. Mean β-glucan content increased from 53.9 to 59.9 g kg^{-1} in one population, and from 63.5 to 66.0 g kg^{-1} in the other, following selection. Genetic variance of β-glucan content decreased by 9 to 22% following selection, but heritability for β-glucan content did not change significantly. Heritability estimates ranged from 0.80 to 0.85 on a line mean basis. Additive variance was the only substantial component of genetic variance. Some experimental lines had significantly greater β-glucan content than the best check cultivars and lines. Phenotypic selection for greater groat β-glucan content will be effective for developing cultivars with elevated β-glucan contents.

Serum cholesterol levels are strongly related to coronary heart disease risk in humans (Mayes, 1990; Shekelle et al., 1981). Greater levels of high-density lipoprotein cholesterol are related to lower incidence of coronary heart disease, whereas greater levels of low-density lipoprotein cholesterol are associated with greater levels of coronary heart disease (Castelli et al., 1986; Stamler, 1979; Miller et al., 1977). Soluble fiber has the potential to reduce serum cholesterol levels in humans (Shinnick et al., 1991; Van Horn et al., 1988; Jenkins et al., 1975). Oat bran and oatmeal have been intensively studied as sources of dietary fiber for human diets (Sacks, 1991). Oat soluble fiber, when added to the daily diet, tends to lower serum low-density lipoprotein cholesterol and total blood cholesterol, particularly in individuals with initially greater levels of serum cholesterol (Welch, 1995; Ripsin et al., 1992; Shinnick et al., 1991; Anderson et al., 1984). This results in a reduction of the risk of coronary heart disease. The (1→3),(1→4)-β-D-glucan (β-glucan) has been identified as the active component of soluble fiber that lowers serum cholesterol (Davidson et al., 1991; Klopfenstein and Hoseney, 1987).

β-Glucan is a cell wall polysaccharide found in seeds of the Gramineae (Stinard and Nevins, 1980; Nevins et al., 1978). Among cereals, oat and barley (*Hordeum vulgare* L.) have the greatest concentrations of β-glucan (Wood, 1994; Åman and Hesselman, 1985; Prentice et al., 1980), but oat generally has a larger proportion of soluble β-glucan (Lee et al., 1997). In oat and barley grains, β-glucan is found mainly in the endosperm and the subaleurone layer (Wood, 1993; Wood et al., 1983). Lim et al. (1992) reported that the groat β-glucan content ranged from 38 to 61 g kg^{-1} among 102 oat lines, including commercial cultivars, experimental lines, and accessions of the wild, interfertile oat relative *A. sterilis* L.

Oat β-glucan content is a polygenic trait under the control of genes with mainly additive effects (Holthaus et al., 1996; Kibite and Edney, 1998). Heritability estimates for β-glucan content have ranged from 0.27 to 0.58 (Holthaus et al., 1996; Humphreys and Mather, 1996; Kibite and Edney, 1998). β-Glucan content is affected by environmental factors, including soil nitrogen level and precipitation (Brunner and Freed, 1994; Humphreys et al., 1994; Peterson, 1991; Peterson et al., 1995; Welch et al., 1991). Although genotype × environment interaction sometimes is a significant source of variation for β-glucan content, the ranking of genotypes is generally consistent over environments (Peterson et al., 1995; Brunner and Freed, 1994; Lim et al., 1992; Saastamoinen et al., 1992).

The inheritance of β-glucan content and the availability of technology developed to measure the trait rapidly by flow injection analysis (FIA) (Jørgensen, 1988) or near infrared reflectance spectrophotometry (NIRS) (Osborne et al., 1983) make possible the improvement of oat β-glucan content through phenotypic selection. The development of oat cultivars with greater groat β-glucan contents should increase the nutritional and economic value of the oat crop.

The objectives of this study were (i) to develop two genetically broad-based oat populations from parents with greater levels of β-glucan content and to conduct phenotypic selection for greater groat β-glucan content in these populations, (ii) to determine the progress from phenotypic selection of individual S_0 plants for greater β-glucan content, and (iii) determine if selection resulted in changes in genetic variance and heritability for β-glucan content, and to determine if additive variance is the predominant component of genetic variance for β-glucan content.

C.T. Cervantes-Martinez, Universidad Autonoma Chapingo, Carretera Mexico-Texcoco km 38.5, Texcoco, Edo. de Mex., Mexico 56230; K.J. Frey, Dep. of Agronomy, Iowa State Univ., Ames, IA 50011; P.J. White, Dep. of Food Science and Human Nutrition, Iowa State Univ., Ames, IA 50011; D.M. Wesenberg, USDA-ARS, National Small Grains Germplasm Research Facility, P.O. Box 307, Aberdeen, ID 83210; J.B. Holland, USDA-ARS Plant Science Research Unit, Dep. of Crop Science, Box 7620, North Carolina State Univ., Raleigh, NC 27695-7620. Received 25 Feb. 2000. *Corresponding author (james_holland@ncsu.edu).

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Abbreviations: BLUP, best linear unbiased predictor; FIA, flow injection analysis; NIRS, near infrared reflectance spectroscopy; PI, plant introduction; REML, restricted maximum likelihood.

Table 1. Oat experimental lines and cultivars used to develop the BG1C0 population.

Line or cultivar	Pedigree and source
D831-1-571	Lang/Y22-15-9, Iowa State University
D921-255	Lang/Noble, Iowa State University
H52-5	B525-336/Y247-2//Ogle, Iowa State University
H87-7-4	B709-98-1/Porter, Iowa State University
H730-12	Y22-15-9/Y341-41//Ogle/3/IL75-5681, Iowa State University
Hazel	Coker 227//Clintford/Portal, University of Illinois
IL82-2154	Ogle/L75-5681, University of Illinois
IL85-6183	IL79-3649/IL79-6637, University of Illinois
Marion	Agriculture and Agri-Food Canada, Sainte-Foy, Quebec
MN88156	WI1961-1/Noble/5/Dal/3/Garland/Burnett//Diana/CI8344/4/Noble, University of Minnesota
MN86226	MN79215//Froker/Dal/2/Porter, University of Minnesota
MN88231	WI1961-1/Noble/5/Dal/3/Garland/Burnett//Diana/CI8344/4/Noble, University of Minnesota
MO07929	Bates/2/Pettis/Florida 5000, University of Missouri
NO4-8	Newman*3/Fidler//Kenyan Introduction/3/Tibor/Dumont, Agriculture and Agri-Food Canada, Ottawa
NO5-1	Complex pedigree involving CI2113, AC Percy, Donald, and Dumont, Agriculture and Agri-Food Canada, Ottawa
NO10-1	Newman//Tibor/Dumont, Agriculture and Agri-Food Canada, Ottawa
NO11-1	Complex pedigree involving CI2113, Tibor, and Dumont, Agriculture and Agri-Food Canada, Ottawa
NO20-1	Complex pedigree involving CI2113, Tibor, and Dumont, Agriculture and Agri-Food Canada, Ottawa
O5406...380	Complex pedigree involving Tibor, Marion, and an unlicensed Ottawa hull-less selection, Agriculture and Agri-Food Canada, Ottawa
OA926-2	Ogle*4/Dumont//Donald*4/Dumont, Agriculture and Agri-Food Canada, Ottawa
Premier	WI1961-1/Noble, University of Minnesota
P7869D1-5-3-2-10-1	P7135/2/P70459/P73109, Purdue University
Starter	Dal/3/MN67231/2/Diana/CI8344/4/Noble, University of Minnesota

MATERIALS AND METHODS

Two base populations, BG1C0 and BG2C0, were developed in which to test the effects of selection for greater β-glucan content.

Development of the BG1C0 Base Population

The BG1C0 population was developed by intermating 23 oat breeding lines and commercial cultivars chosen for their greater β-glucan content or good agronomic characteristics (Table 1), followed by an additional generation of intermating unrelated F_1 plants. A third generation of random mating among F_1 plants resulted in 97 crosses. The 1665 S_0 plants obtained from these 97 crosses constituted BG1C0, the base population for selection.

Selection within BG1CO for Greater β-Glucan Content

In 1992, at Aberdeen, ID, 1665 S_0 plants (from two to 38 per cross) from BG1C0 were grown as spaced plants in the field. S_0 plants from the same cross made to obtain the BG1C0 seed represented a full-sib family. The S_1 seed of each S_0 plant was harvested individually and analyzed for groat β-glucan content via automated FIA as described by Lim et al. (1992). The groat β-glucan content of each plant was estimated by the mean of three subsamples and three individual measurements per subsample. The 40 C_0 $S_{0:1}$ lines with greatest β-glucan content represented 21 full-sib families and were selected to be parents of the BG1C1 population. The 40 parent lines were intermated in the greenhouse in spring of 1993 by crossing each line to approximately six others at random to obtain 116 single crosses, which together constituted the BG1C1 population. In 1993, 582 S_0 plants representing the BG1C1 population were grown as spaced plants in the field at Aberdeen, ID. S_0 plants from each cross made to form BG1C1 represented a full-sib family. Seed was harvested from each plant individually to form 582 $S_{0:1}$ lines.

Development of the BG2C0 Base Population

The BG2C0 base population was developed primarily by crossing selected lines from the BG1C1 population with lines from a population (BGPI) possessing germplasm from unadapted plant introductions (PIs) with greater β-glucan content. The BGPI population was used as a donor for potentially unique alleles for greater β-glucan content. It was developed by mating S_0 plants from 10 crosses with at least 25% PI parentage (Table 2) to the same 40 $S_{0:1}$ lines selected from BG1C0 that were also used as parents of BG1C1.

The choice of lines from the BG1C1 and BGPI populations to use as parents for the second base population was made on the basis of β-glucan contents of S_0 plants grown at Aberdeen in 1993. A total of 951 S_0 plants (from one to ten per full sib family) representing the BG1C1 and BGPI populations combined were evaluated in a common experiment. Grain was harvested from each plant individually and analyzed for β-glucan content via FIA.

Thirty-eight $S_{0:1}$ lines representing 31 full-sib families from the BG1C1 population and seven lines from the BGPI population were selected to be used as parents of BG2C0 because of their greater β-glucan contents. In addition, 12 high β-glucan experimental lines and the cultivar Marion also were selected to be parents for the BG2C0 population, giving a total of 50 parents. The 50 parent lines were intermated in the greenhouse in spring of 1994 by crossing each line to approximately 10 others at random to obtain 248 crosses. The S_0 seed of these crosses constituted the BG2C0 population. The BG2 population is related to the BG1 population, but differs from it by the inclusion of PI germplasm and of germplasm from only selected lines of BG1C0 and BG1C1.

Selection within BG2C0 for Greater β-Glucan Content

In 1994, at Aberdeen, ID, 916 BG2C0 S_0 plants (from one to five per cross) were grown as spaced plants. S_0 plants from the same cross made to form the BG2C0 seed represented a full-sib family. Each S_0 plant was harvested individually and analyzed for groat β-glucan content via FIA. Selection was first performed among 248 full-sib families by selecting the 50 families with greatest mean β-glucan content. Then within-family selection was practiced by choosing the line with the greatest β-glucan content within each of the selected families to be a parent of the BG2C1 population. The selected lines were intermated in the greenhouse in spring of 1995 by crossing each line to approximately 10 others at random. S_0 seeds from 250 crosses among these parent lines constituted the

Table 2. Crosses involving plant introductions of oat that contributed to BGPI and BG2C0 populations.

Cross	Pedigree
IA92001	PI361884 (from Romania)//PI142689 (from Turkey)/IL85-6183
IA92002	PI412928 (from South Africa)//PI412689 (from Turkey)/IL85-6183
IA92003	PI504601 (from Penn. State Univ.)//PI411649 (*A. sterilis* from Eritrea)/MO07929
IA92004	PI504611 (from Penn. State Univ.)//PI411649 (*A. sterilis* from Eritrea)/MO07929
IA92005	PI361886 (from Romania)//PI309123 (*A. sterilis* from Israel)/H87-24
IA92006	PI504593 (from Penn. State Univ.)//PI309123 (*A. sterilis* from Israel)/H87-24
IA92007	PI502955 (from Iowa State Univ., 25% of parentage is PI298129, *A. sterilis* from Israel)//PI295903 (*A. sterilis* from Israel)/IA91539
IA92008	Marion//PI295903 (*A. sterilis* from Israel)/IA91539
IA92009	PI309598 (*A. sterilis* from Israel)//Premier
IA92010	PI412418 (*A. sterilis* from Israel)//Premier

BG2C1 population. The BG2C1 population was grown as spaced plants at Aberdeen, ID, in the summer of 1995. Each plant was harvested individually to form 1471 $S_{0:1}$ lines.

Field Evaluation

Experimental and check oat lines were evaluated in field experiments in 1996 and 1997. The experimental design was a sets within replications design. Each replication had five sets. Each set of 100 entries was arranged as a randomized complete block with two replications at each environment. The experiment was grown at the Agronomy and Agricultural Engineering Field Research Center near Ames, IA, on a Nicollet loam soil (fine-loamy, mixed, mesic Aquic Hapludoll) and the Northeast Research Center near Nashua, IA, on a Readlyn loam soil (fine-loamy, mixed, mesic Aquic Hapludoll) in both years.

The treatment design consisted of five populations (BG1C0, BG1C1, BG2C0, BG2C1, and BGPI). Each population was represented by 100 $S_{0:1}$ lines. The family structure created during population development was maintained; lines that were derived from the same cross were members of a common full-sib family. Two lines were randomly chosen from each of 50 randomly chosen full-sib families, resulting in a sample of 100 lines from each population. An exception to this was that only 33 families (66 lines) from the BG1C1 population and only 17 families (34 lines) from the BGPI population were evaluated. Families were randomly assigned to five sets such that each set received both lines from 10 families from each of the BG1C0, BG2C0, BG2C1 populations; 4 to 10 families (8–20 lines) from the BG1C1 population; and zero to six families (0–12 lines) from the BGPI population. There were a total of 80 experimental lines per set. Twenty check or parent line entries also were included in each set. Checks included seven commercial oat cultivars (Don, Marion, Hazel, Premier, Ogle, Starter, and Noble), which were included in each set in duplicate. Each set also included five of the original parental lines of BG1C0 (Table 1) or BGPI (PI412928, PI361884, PI361886, PI502955, PI504593, or PI504601), which were assigned to sets at random. Each set also included a high β-glucan experimental line, IAN979-5-2, to make a total of 100 entries per set.

Field plots consisted of hills of 20 seeds planted on a grid 0.3 m apart. Each experiment was surrounded by two rows of hills of a common check cultivar to provide competition to peripheral plots. Weeds were controlled manually. Plots were sprayed with the systemic fungicide Bayleton [1-(4-chlorophenoxy)-3.3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone] to protect them from crown rust [*Puccinia coronata* Corda] infection. To have sufficient seed for spectrophotometry and chemical analysis, the grain from both replications of each entry within a location was bulked together, mixed thoroughly, and the β-glucan content of a representative portion of each sample was estimated.

Grain samples were dehulled with an air pressure dehuller to obtain approximately 8 g of groats. The β-glucan content of each groat sample was determined with a near-infrared reflectance spectrophotometer (NIRS). The β-glucan value for each sample was the mean of three measurements. To calibrate the prediction equation for β-glucan content for each evaluation year, β-glucan contents of 92 samples from the 1996 evaluation and 95 samples from the 1997 evaluation (representing approximately 10% of the total number of samples from each year) also were measured with automated FIA as described by Lim et al. (1992). The calibration samples were selected on the basis of NIRS spectral features to best represent the spectral variability of the whole set of samples. A β-glucan determination for a sample chosen for the calibration equation was the mean of nine values: three subsamples were taken from each sample and three FIA measurements were obtained for each subsample. The prediction equations for each year of evaluation were developed using modified partial least squares regression (Benson, 1986; Aastveit and Martens, 1986). Briefly, 70 to 80% of the calibration samples (for which both NIRS and FIA measurements were available) were assigned at random to a training data set, whereas the remaining samples were assigned to a test data set. Partial least squares regression was applied to the training set. Models obtained from the training data set were applied to the test data set and the standard errors of cross validation were calculated for each model. The optimal number of latent variables extracted by the partial least squares regression method was determined by choosing the model that minimized the standard error of cross validation. Partial least squares regression was then applied to the entire calibration data set, retaining the optimal number of latent variables determined by test set validation. This model was then used to predict β-glucan values for the remaining NIRS samples.

Statistical Analysis

To compare population means, an analysis was performed by Proc MIXED of SAS (Littell et al., 1996), considering population as a fixed effect factor and all other factors (environment, set, environment × set, population, environment × population, set × population, environment × set × population, genotype within set × population, environment × genotype within set × population, and residual) as random. The analysis was performed on values for β-glucan content of each bulk of grain representing an entry-environment combination. The residual variance was due to check entries repeated within sets and environments. Best linear unbiased predictors (BLUPs) for genotypes were computed as linear functions of fixed and random effect estimates. The BLUP for Genotype k within Population j and Set i was calculated as:

$$BLUP_{ijk} = \mu + S_i + \overline{ES}_{.i} + P_j + SP_{ij} + \overline{ESP}_{.ij} + G(SP)_{ijk},$$

where m is the overall mean; S_i is the effect of Set i; $\overline{ES}_{.i}$ is the mean of environment × set interaction effects involving

Set i averaged over all environments; P_j is the effect of Population j; SP_{ij} is the effect of the interaction of Set i and Population j; $\overline{ESP}_{.ij}$ is the mean of environment × set × population interaction effects involving Set i and population j averaged over all environments; and $G(SP)_{ijk}$ is the effect of the Genotype k within Set i and Population j. The sum of population × environment interaction effects over all environments for a specific population is zero, therefore this term does not contribute to the genotypic BLUP. Standard errors for comparisons of BLUPs within the same set were obtained using "estimate" statements in Proc MIXED (Littell et al., 1996).

The components of variance within each population were estimated by the REML method (Searle, 1971) using Proc MIXED of SAS (Littell et al., 1996). Each population was analyzed independently, considering all effects (environment, set, environment × set, family within set, environment × family within set, line within family within set, and residual environment × line within family within set) except the overall mean to be random. Significance tests of the components of variance were obtained with the likelihood ratio test, which assumes that the difference between the –2 REML log-likelihood of the full model and the reduced model without the component of variance in question has a chi-square distribution with one degree of freedom (Self and Liang, 1987; Littell et al., 1996).

We were able to classify lines according to which full-sib family (or cross) they belonged because of the family structure within populations. Therefore, we were able to estimate components of variance due to full-sib family and due to $S_{0:1}$ line within family for each population. This partitioning of the total genetic variance into among- and within-full-sib family variation allowed us to test the hypothesis that additive genetic variance was the sole component of genetic variance, as follows. Genetic expectations of the family and line within family variance components were derived following Cockerham (1971, 1983). The genetic expectations for these variance components in the BG1C0 population were different from the other populations because the parents of the BG1C0 population were non-inbred S_0 plants, whereas the parents of BG1C1, BG2C0, and BG2C1 were partially inbred S_1 plants. The genetic expectancies of family and $S_{0:1}$ line within family variance components of the BG1C0 population are:

$$\sigma^2_{family} = (1/2)\sigma^2_A + (1/32)\sigma^2_D + (1/2)D_1 + (1/16)D_2 + (1/4)\sigma^2_{AA} + (1/32)\sigma^2_{AD} + (1/256)\sigma^2_{DD}$$

and

$$\sigma^2_{line(family)} = (1/2)\sigma^2_A + (3/16)\sigma^2_D + (1/2)D_1 + (1/16)D_2 + (3/4)\sigma^2_{AA} + (7/32)\sigma^2_{AD} + (15/256)\sigma^2_{DD},$$

where σ^2_A is the additive variance, σ^2_D is the dominance variance, D_1 is the covariance between additive effects and homozygous dominant effects, D_2 is the variance of homozygous dominant effects, and σ^2_{AA}, σ^2_{AD} and σ^2_{DD} are the additive × additive, additive × dominant and dominant × dominant epistatic variances, respectively (Nyquist, 1991). The expectations of the variance components of family and line within family of the BG1C1, BG2C0 and BG2C1 populations are:

$$\sigma^2_{family} = (3/4)\sigma^2_A + (9/64)\sigma^2_D + (3/4)D_1 + (3/32)D_2 + (9/16)\sigma^2_{AA} + (27/256)\sigma^2_{AD} + (81/4096)\sigma^2_{DD}$$

$$\sigma^2_{line(family)} = (1/4)\sigma^2_D + (7/64)\sigma^2_D + (1/4)D_1 + (1/32)D_2 + (7/16)\sigma^2_{AA} + (37/256)\sigma^2_{AD} + (175/4096)\sigma^2_{DD}.$$

Under the hypothesis that additive genetic variance is the only substantial component of the genetic variance, the following equalities are true: $\sigma^2_{family} - \sigma^2_{line(family)} = 0$ for BG1C0 and $\sigma^2_{family} - 3\sigma^2_{line(family)} = 0$ for the BG1C1, BG2C0, and BG2C1 populations. These equations were tested assuming asymptotic normality of the variance component estimates (Self and Liang, 1987; Searle, 1971).

Heritability on a line mean basis was estimated as:

$$H^2 = (\hat{\sigma}^2_{family} + \hat{\sigma}^2_{line(family)})/[\hat{\sigma}^2_{family} + \hat{\sigma}^2_{line(family)} + ((\hat{\sigma}^2_{env\times family} + \hat{\sigma}^2_{env\times line(family)})/4)].$$

Heritability on a family mean basis was estimated as:

$$H^2 = \hat{\sigma}^2_{family}/[\hat{\sigma}^2_{family} + (\hat{\sigma}^2_{line(family)}/2) + (\hat{\sigma}^2_{env\times family}/4) + ((\hat{\sigma}^2_{env\times line(family)})/8)].$$

Approximate standard errors of heritability estimates were obtained by means of the delta method (Lynch and Walsh, 1997).

RESULTS AND DISCUSSION

Prediction Equation Validation

The coefficient of determination (R^2) of the NIRS prediction equation for β-glucan content was 0.76 for the 1996 evaluation and 0.80 for 1997. The standard error of calibration was 4.7 g kg^{-1} for 1996 and 3.8 g kg^{-1} for 1997. The standard error of FIA laboratory measurements was 3.3 g kg^{-1} in 1996 and 0.6 g kg^{-1} in 1997. The precision of FIA measurements was much greater in 1997 than in 1996, but this resulted in only a small improvement in the precision of the regression equation.

Response to Selection

Selection resulted in significant increases in mean β-glucan content in both populations (Table 3). The mean β-glucan content of the BG1 population changed from 53.9 g kg^{-1} in C0 to 59.9 g kg^{-1} in C1, an increase of 11% of the unselected population mean. A smaller increase of 4% was observed in the BG2 population,

Table 3. Mean groat β-glucan contents of the initial (C0) and selected (C1) generations of two broad-based oat populations (BG1 and BG2); the wild germplasm donor population (BGPI); parental lines; and check cultivars averaged across environments.

Population or group	β-Glucan content
	g kg^{-1}
BG1C0	53.9a†
BG1C1	59.9b
BG2C0	63.5c
BG2C1	66.0d
BGPI	56.9e
Check cultivars	55.3a
Parental lines	54.7a

† Means followed by the same letter were not significantly different at $P = 0.05$, based on LSD.

Table 4. Best linear unbiased predictors (BLUPs) of β-glucan content (and rank) of experimental oat lines with greatest β-glucan BLUPs and check cultivars from set one.

Population	Line	β-Glucan content BLUP
		g kg^{-1}
BG2C0	IA94031-7	71.4 (1)†a‡
BG2C1	IA95172-1	68.3 (2)a
BG2C0	IA94031-6	68.2 (3)a
BG2C1	IA95181-6	68.2 (4)a
BG2C1	IA95109-2	68.1 (5)a
BG2C1	IA95029-3	67.9 (6)a
BG2C1	IA95148-3	67.6 (7)a
BG1C1	IA95109-3	67.5 (8)a
BG1C0	IA94190-10	67.0 (9)ab
BG2C1	IA95148-1	67.0 (10)ab
Checks	IAN979-5-2‡	62.8 (20)bc
	Premier	60.8 (35)cd
	Marion	57.7 (54)de
	Starter	56.2 (62)e
	Hazel	51.1 (79)f
	Noble	50.5 (83)f
	Don	48.9 (86)f
	Ogle	48.5 (88)f
Mean of all entries		58.5

† Rank of genotype's BLUP for β-glucan content within set.
‡ BLUPs followed by the same letter are not significantly different at $P = 0.05$, based on standard errors of BLUP comparisons.

which changed from 63.5 g kg^{-1} in C0 to 66.0 g kg^{-1} in C1. The mean β-glucan content of check cultivars was not significantly different from the BG1C0 or parental line means, and was significantly lower than the BG1C1, BG2C0, BG2C1, and BGPI means (Table 3).

Lines from the BG2C0 and BG2C1 populations generally had greater β-glucan content than lines from BG1C0 and BG1C1. This was because the BG2 population was developed in part from lines selected for greater β-glucan content from the BG1C1 population. In set one, for example, two lines from BG2C0 and six lines from BG2C1 had significantly greater β-glucan content BLUPs than the check with greatest β-glucan content, experimental line IAN979-5-2 (Table 4). The 10 lines with greatest β-glucan content BLUPs in set one had significantly greater β-glucan percentage than all of the commercial checks (Table 4). Similar trends were observed in the other four sets.

Selections were made on the basis of β-glucan content of plants grown in Idaho (a favorable oat-growing region, Holland et al., 2000), whereas evaluation of response to selection was performed in Iowa (a more stressful environment for oat, Holland et al., 2000).

Table 6. Heritability estimates (and their standard deviations) for β-glucan content within each of four experimental oat populations.

Heritability estimator	BG1C0	BG1C1	BG2C0	BG2C1
$S_{0:1}$ Line mean-basis†	0.84 (0.03)	0.85 (0.04)	0.83 (0.03)	0.80 (0.04)
Full-sib family mean-basis‡	0.56 (0.12)	0.77 (0.08)	0.72 (0.08)	0.70 (0.09)

† Heritability on an $S_{0:1}$ line mean-basis estimated as: $(\sigma^2_{family} + \sigma^2_{line(family)})/[\sigma^2_{family} + \sigma^2_{line(family)} + ((\sigma^2_{env\times family} + \sigma^2_{env\times line(family)})/4)]$.
‡ Heritability on a full-sib family mean-basis estimated as: $\sigma^2_{family}/[\sigma^2_{family} + (\sigma^2_{line(family)}/2) + (\sigma^2_{env\times family}/4) + ((\sigma^2_{env\times line(family)})/8)]$.

Therefore, the observed gains in population means demonstrated that β-glucan content was sufficiently stable as to permit substantial progress from breeding to be observed across diverse environments.

Variance Component and Heritability Estimates

The family, family × environment, and line within family components of variance were significant in each population (Table 5). The family variance component was larger than the family × environment interaction variance within each population. Heritability estimated on a line mean basis ranged from 0.80 to 0.85 and heritability estimated on a family mean basis ranged from 0.56 to 0.77 (Table 6).

Since genetic variance depends on gene frequency, selection may cause changes in the components of variance (Falconer and Mackay, 1996). We observed reductions of 9% and 22% in the genetic variance for β-glucan content in BG1 and BG2, respectively, after selection for greater β-glucan content (Table 5). Nevertheless, there was no evidence for a significant reduction in heritability due to selection in either population (Table 6).

The contrasts σ^2_{family} vs $\sigma^2_{line(family)}$ for BG1C0 and σ^2_{family} vs $3\sigma^2_{line(family)}$ for the BG1C1, BG2C0, and BG2C1 populations were not significant (Table 5), indicating that we could not reject the null hypothesis that additive genetic variance was the only substantial component of the genetic variance. This result agrees with earlier reports that oat β-glucan is a polygenic trait under the control of genes with mainly additive effects (Kibite and Edney, 1998; Holthaus et al., 1996). Assuming that additive genetic variance is the sole genetic component of variance, the total genetic variance in a random mated population is equal to $\sigma^2_{family} + \sigma^2_{line(family)}$ (Table 5).

Table 5. Variance component estimates (and their standard deviations) and test for non-additive genetic variance for β-glucan content from each of four experimental oat populations.

Source	Population			
	BG1C0	BG1C1	BG2C0	BG2C1
	g^2 kg^{-2}			
Family	7.6** (3.1)	10.6** (3.7)	12.6** (3.8)	10.3** (3.4)
Family × Environment	4.8** (1.2)	2.4* (1.1)	6.2** (1.5)	7.5** (1.6)
Line(Family)	7.7** (2.0)	3.2** (1.3)	4.7** (1.5)	3.3** (1.3)
Environment × Line(Family)	7.0** (0.9)	7.2** (1.1)	8.3** (1.1)	7.0** (1.0)
Test for non-additive genetic variance				
$\sigma^2_{family} - \sigma^2_{line(family)}$	−0.1	NA†	NA	NA
$\sigma^2_{family} - 3\sigma^2_{line(family)}$	NA	1.1	−1.6	0.3

* Indicates significance at $P = 0.05$.
** Indicates significance at $P = 0.01$.
† Not applicable.

CONCLUSION

Phenotypic selection of field-grown S_0 plants for greater β-glucan content increased the mean of this trait in the BG1 and BG2 populations by 11 and 4%, respectively. The intermediate heritability, the predominantly additive inheritance, the significant genetic variability of the trait, and the availability of instruments to rapidly measure groat β-glucan concentration allowed substantial improvements to be made with single-plant phenotypic selection. Furthermore, β-glucan content is sufficiently stable that selections made in favorable Idaho environments resulted in improvements realized in more stressful Iowa environments. The germplasm lines with elevated β-glucan content developed from this program should prove useful as breeding parents for cultivar development programs.

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CROP PHYSIOLOGY & METABOLISM

Winter Hardiness, Root Physiology, and Gene Expression in Successive Fall Dormancy Selections from 'Mesilla' and 'CUF 101' Alfalfa

S. M. Cunningham, J. A. Gana, J. J. Volenec,* and L. R. Teuber

ABSTRACT

Fall dormancy is positively associated with alfalfa (*Medicago sativa* L.) winter survival, but the physiological bases for this association are not understood. Our objective was to determine how incremental changes in fall dormancy due to genetic selection influenced autumn height and winter survival, root physiology, and expression of a cold acclimation responsive gene family. Seed from each of three cycles of selection for contrasting (greater or less) fall dormancy using 'Mesilla' and 'CUF 101' as parents were planted in rows in the field (Starks-Fincastle, fine-silty, mixed, mesic, Aeric Ochraqualf) in West Lafayette, IN, in May 1997 and 1998. Plant height was measured in October and roots were sampled in December. Plant survival was determined in March of the year following seeding. Fall dormancy (reduction in shoot height in October) increased in a linear manner over the three cycles of selection for both Mesilla and CUF 101. A positive linear relationship was observed between fall height and winter injury in both years. Root sugar and protein concentrations increased as fall dormancy increased in populations derived from both Mesilla and CUF 101. Expression of the cold acclimation-responsive gene, RootCAR1, was positively associated with winter survival, and may be useful as a molecular marker for identifying winter hardy plants among semi-dormant or nondormant alfalfa germplasm in December of the seeding year.

Fall dormant alfalfa cultivars produce short, decumbent shoots in autumn, whereas fall nondormant plants possess tall, upright shoots in autumn (Smith, 1958; Barnes et al., 1979; Sheaffer et al., 1992; Cunningham et al., 1998). Non-fall dormant alfalfa cultivars are desirable because nondormant plants produce more herbage in autumn, resume shoot growth earlier in spring, and initiate shoot regrowth quickly after harvest in summer (Zaleski, 1954; Busbice and Wilsie 1965). Once shoot initiation occurs, shoot elongation rate of nondormant alfalfa is up to twice that of fall-dormant germplasm pools, and results in large differences in leaf area expansion and mass per shoot (Volenec, 1985), factors known to be correlated with high forage yield (Volenec et al., 1987).

The major constraint preventing widespread use of nondormant alfalfas in temperate regions is their poor winter hardiness. Sheaffer et al. (1992) examined in spring-planted alfalfa the interactions between seeding-year harvest management, fall dormancy, and winter survival in Minnesota. They reported that fall dormancy influenced alfalfa winter hardiness more than any other feature they measured. Nondormant alfalfa cultivars died during winter irrespective of harvest management or location, whereas fall-dormant cultivars had good winter survival. These findings are supported by other studies (Smith, 1961; Barnes et al., 1979; Stout, 1985; Stout and Hall, 1989), and have resulted in fall dormancy routinely being used to predict alfalfa winter hardiness.

The mechanisms controlling the close positive association between fall dormancy and winter hardiness are not clearly understood. Genetic differences in alfalfa winter survival have been associated with several physiological changes in overwintering organs. Accumulation of starch and sugar in taproots has been positively associated with alfalfa winter survival (Graber et al., 1927; Grandfield, 1943; Smith, 1964). Accumulation of soluble sugars in roots is thought to enhance tolerance to low temperatures and other stresses associated with winter (Bula et al., 1956; Ruelke and Smith, 1956). Castonguay et al. (1995) and Castonguay and Nadeau (1998) recently reported that the accumulation of raffinose and stachyose was more closely associated with winter survival than was accumulation of starch or sucrose. Other studies indicate that N-containing compounds also accumulate in roots during winter hardening, and serve as a source of N when shoot growth is initiated in spring and for regrowing shoots after harvest (Volenec et al., 1991, 1996; Hendershot and Volenec, 1993; Avice et al., 1996). Differential gene expression and accumulation of their gene products occur during cold acclimation, and these changes also have been positively associated with ge-

S.M. Cunningham, J.A. Gana, and J.J. Volenec, Dep. of Agronomy, Purdue Univ., West Lafayette, IN 47907-1150; L.R. Teuber, Dep. of Agronomy and Range Science, Univ. of California, Davis, CA 95616-8515. Contribution from the Purdue Univ. Agric. Exp. Stn., Journal Series no. 16320. Received 11 Aug. 2000. *Corresponding author (jvolenec@purdue.edu).

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Abbreviations: LSD, least significant difference; PMSF, phenyl methyl sulfonyl fluoride; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; TNC, total nonstructural carbohydrate.