Arabinoxylan content of hard winter and spring wheats of the U.S. Pacific Northwest

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ABSTRACT

The development of high quality wheat (*Triticum aestivum* L.) cultivars depends on a thorough understanding of the genetic and environmental influences on the constituents of grain. Arabinoxylans are important albeit quantitatively minor constituents of wheat grain; they can interact with large weight ratios of water and participate in oxidative cross-linking and gel formation. In this study, 25 hard winter and 25 hard spring wheat genotypes from breeding programs in the U.S. Pacific Northwest were analyzed for water-soluble and total arabinoxylan contents. Each genotype set was grown in three environments. There were significant differences among water-extractable (WE-AX) and total (TO-AX) arabinoxylan contents (G, E, and G*E model R^2s 0.64-0.96). WE-AX genotype mean content ranged about 2-fold, from 0.39 to 0.81% for winter, and 0.48 to 0.92% for spring wheat genotypes. TO-AX genotype mean content ranged from 3.1 to 4.0% for winter and 3.9 to 4.7% for spring genotypes. Type III Sums of Squares *F*-ratios for 'genotype' were highly significant ($P<0.0001$) for both AX fractions of both winter and spring genotypes. 'Hollis' spring wheat had the highest WE-AX content and 'WQL9HALP' spring wheat (a hard NIL to 'Alpowa') had the highest TO-AX content. Repeatability estimates were 0.71 and 0.89 for WE-AX, winter and spring; and 0.30 and 0.62 for TO-AX, winter and spring genotypes, respectively. These preliminary results indicate that there is sufficient repeatable genetic variation to improve hard winter and spring wheat cultivars for AX contents.

INTRODUCTION

Arabinoxylans are an important group of non-starch polysaccharides present in wheat grain. Also known as pentosans, these heteromorphic, large polymers play an important role in end-use quality, primarily through their interaction with water and ability to cross link other arabinoxylan molecules and proteins. Previous work (Finnie et al. 2006) found that significant genetic differences were present among soft white winter and soft white spring wheat cultivars and breeding lines in the U.S. Pacific Northwest. The present paper describes the arabinoxylan content of hard winter and hard spring wheats from this same region.

Arabinoxylans of wheat grain are generally categorized according to their solubility at room temperature in water. In other words, that fraction of arabinoxylan that is freely soluble in water is referred to as water extractable (WE-AX); the fraction that is water insoluble and associated with cell walls is referred to as water insoluble-AX (WI-AX). The water insoluble fraction is usually determined by subtracting the WE-AX fraction from the measurement of the total arabinoxylan (TO-AX). Arabinoxylans are comprised of a xylan backbone variously substituted with arabinofuranosyl moieties. Additionally, ferulic acid may be esterified at the O-5 position of arabinose and available for cross-linking and formation of oxidative gels.

MATERIALS AND METHODS

Hard red and hard white wheat samples were obtained from an ongoing genotype and environment study conducted by the USDA-ARS Western Wheat Quality Laboratory. Depending on growth habit (i.e., winter or spring), the samples were organized and grown in two sample sets by the Washington State University Cereal Variety Testing (WSUCVT) Program, and harvested in 2006. An equal number (25 each) of winter and spring wheat varieties were included. Genotypes included commercial cultivars and advanced breeding lines. Each set of genotypes was grown at three locations.

Standardized milling and baking evaluations were conducted at the WWQL using AACC International (2000) Methods or others described at: http://www.wsu.edu/~wwql. Arabinoxylan determination of finely ground whole grain meal followed a colorimetric method described by Douglas (1981). The method hydrolyzes arabinoxylan to its constituent pentose sugars that are then reacted with phloroglucinol. Absorbance readings ($\Delta A_{552-510}$) were calibrated against a xylose standard and expressed as a percentage by weight. WE-AX was determined from a slurry supernatant, TO-AX was determined from an aqueous suspension, and the WI-AX by subtraction.

ANOVA (analyses of variance) of arabinoxylan fractions was conducted using Proc GLM (SAS Institute, Cary, NC) separately for the winter and spring wheat genotype sets using a factorial model with genotypes (G), environments (E) and the G x E interaction term. Genotype was considered a fixed, and environment was considered a random effect. Model component *F*-tests were performed using Type III mean squares. The environment main effect was tested using the genotype x environment interaction as the error term by specifying such as 'e' in the 'random' statement. Repeatability estimates were calculated as 1-(Mean Square_{G*E} / Mean Square_{G}) using Type III Mean Squares. Genotype mean separation was determined using Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$.

RESULTS AND DISCUSSION

There were significant differences among water-extractable (WE-AX) and total (TO-AX) arabinoxylan contents of the hard wheat grain samples. ANOVA models with G, E, and G*E components returned R^2s of 0.64-0.96. WE-AX genotype mean content ranged about 2-fold from 0.39 to 0.81% for winter genotypes, and 0.48 to 0.92% for spring genotypes. TO-AX genotype mean content ranged from 3.1 to 4.0% for winter, and 3.9 to 4.7% for spring genotypes. Type III Sums of Squares *F*-ratios for 'genotype' were highly significant (P<0.0001) for both AX fractions of both winter and spring genotypes.

ANOVA indicated that genetic variation was similar between the two classes of wheat for both AX fractions. For WE-AX, G and E components were of similar magnitude in terms of contribution to variation. For TO-AX, however, E was much greater than G for the winter wheats, whereas for spring wheats the three environments were not significantly different.

Repeatability estimates were calculated using the ANOVA Type III Mean Squares (Table 1). Repeatability estimates were 0.71 and 0.89 for WE-AX, winter and spring genotypes; and 0.30 and 0.62 for TO-AX, winter and spring genotypes, respectively. The lower value for TO-AX of the spring wheat genotypes reflects the low amount of variation among the three environments.

Table 1. ANOVA Type III Mean Squares and repeatability estimates

Winter Wheat		Spring Wheat	
WE-AX		WE-AX	
G*E	0.0180	G*E	0.0101
Genotype	0.0618	Genotype	0.0940
Repeatability	0.709	Repeatability	0.893
Total AX		Total AX	
G*E	0.2654	G*E	0.0942
Genotype	0.3798	Genotype	0.2454
Repeatability	0.301	Repeatability	0.616

From the two sets of genotypes, those with 'extreme' (highest and lowest) contents of WE- and TO-AX fractions, and the proportion of WE-AX of the TO-AX were identified by ANOVA and DMRT (Table 2). Juniper hard red winter had the highest WE-AX content as well as the highest proportion of WE-AX of TO-AX (23.0%). Breeding lines WA8002 and ID621 had the highest TO-AX contents. ORN980995 was of interest in that it was lowest for all three AX fractions. Among the spring wheats, Hollis hard red spring had the highest WE-AX and highest proportion of TO-AX that was water extractable. For TO-AX, the hard white spring near-isogenic line, WQL9HDALP (Morris and King, 2008), had the highest content. At the other extreme, Alta Blanca had the lowest WE-AX, TO-AX and proportion of TO-AX that was water extractable.

Table 2. Wheat grain arabinoxylan content (%) of extreme high and low hard winter and spring wheat genotypes

Genotype	WE-AX	Total AX	WE/Total
Juniper	0.808 a	3.59 b	23.0 a
ID621	0.711 bc	3.96 a	18.6 b
WA8002	0.706 bc	4.04 a	17.4 b
Eddy	0.401 d	3.70 ab	11.7 d
ORN980995	0.390 d	3.09 c	13.6 c
LSD $_{(0.05)}$	0.059	0.30	1.9
Hollis	0.919 a	4.25 bc	21.6 a
Blanca Grande	0.875 a	4.57 ab	19.3 b
WQL9HDALP	0.769 c	4.70 a	16.4 c
Alta Blanca	0.476 d	3.94 c	12.1 d
LSD $_{(0.05)}$	0.051	0.33	1.7

All grain and quality traits were analyzed for possible correlation with AX fractions. Although a number of correlations were significant, only those that were consistent for both winter and spring wheat sets are presented in Table 3. WE-AX increased with test weight, perhaps a reflection of greater endosperm-to-bran ratio. SKCS hardness standard deviation was negatively correlated with WE-AX. A possible explanation for this result was not immediately apparent. Break flour yield, a measure of kernel hardness and milling performance decreased with increasing WE-AX.

For the WI-AX fraction, increases were correlated with smaller kernels as determined by the SKCS kernel weight and size (diameter) measurements (Table 3). Again, this result may reflect that smaller kernels have a lower endosperm-to-bran ratio.

When WE-AX was examined as a proportion of the TO-AX, consistent and significant correlations were returned for all previous parameters (Table 3). Coefficients were similar for those obtained with WE-AX. Conversely, coefficients tended to change sign compared to the WI-AX results. Of note was the positive correlation with loaf volume, where 6-11% of variation (r^2) was associated with variation in this AX measure, higher proportions of WE-AX being correlated with larger loaf volume. WE-AX was positively correlated with loaf volume among the spring genotypes, whereas among the winter genotypes WI-AX was negatively correlated with loaf volume. Naturally, it will be of interest to measure the AX contents of flour along with some estimate of bran contamination to better resolve the interplay of milling/flour extraction rate and flour/dough performance.

Clearly the results indicate that there are notable genetic differences for AX in wheat grain, and that differences in (ostensibly) molecular structure, cross-linking, etc. confer significant differences (often 2-fold) in solubility. This emerging field of wheat grain quality as related to processing and end-product quality is deserving of further study. The identification of specific biosynthetic or polymer-modifying genes is needed. Also, the formation of oxidative gels via ferulic acid cross-linking adds a further dimension to AX functionality.

Table 3. Correlations of arabinoxylan fractions with grain and quality traits

	WE-AX		WE-AX/To-AX	
Trait	Winter	Spring	Winter	Spring
Test Weight	0.24	0.28	0.26	0.28
SKCS Hard.sd	-0.29	-0.26	-0.35	-0.25
Break Flour Yield	-0.33	-0.30	-0.19	-0.32
Loaf Volume	ns	0.35	0.24	0.34
	WI-AX		WE-AX/To-AX	
Trait	Winter	Spring	Winter	Spring
SKCS Weight	-0.27	-0.26	0.30	0.54
SKCS Size	-0.43	-0.24	0.38	0.61
Loaf Volume	-0.38	ns	--see above--	

$r = 0.22$ significant at $P = 0.05$; $r = 0.29$ significant at $P = 0.01$

REFERENCES

AACC International (2000) Approved Methods of the American Association of Cereal Chemists, 10th ed. AACC International, St. Paul, MN

Douglas SG (1980) A rapid method for the determination of pentosans in wheat flour. *Food Chem* 7:139-145.

Finnie SM, Bettge AD, Morris CF (2006) Influence of cultivar and environment on water-soluble and water-insoluble arabinoxylans in soft wheat. *Cereal Chem* 83:617-623.

Morris CF King GE (2008) Registration of hard kernel puroindoline allele near-isogenic line hexaploid wheat genetic stocks. *J Plant Registrations* 2:67-68.