Effects of genotype and environment on B-glucan and dietary fibre contents in whole grain of barley grown in Brazil

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ABSTRACT

The aim of this study was to determine general quality characteristics of whole grain of advanced lines of barley grown in Brazil and to determine the effects of genotype and growing location on β-glucan and dietary fibre contents. Barley cultivars, designated BRS 225, BRS 195 and MN 743, crop year 2008 and 2009, with three replications in field trial coordinated by the EMBRAPA Trigo/Passo Fundo were used in this research. The barley samples were analyzed in relation to their Total Dietary Fibre (TDF) contents and fractions for moisture and protein (N × 6.25) by using AACC Methods (American Association of Cereal Chemists, 1990). The β-Glucan content was assessed using the McCleary Enzymic Method for barley (McCleary and Codd, 1991). The data was statistically evaluated by System for Analysis and Separation Averages in Agricultural Experiment (SASM-Agri - version 4) and differences between means were located using Scott-Knott’s test (p<0.05). There were significant differences among the barley genotypes and different locations and crop years in terms of β-glucan, total dietary fibre (TDF), soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) content (p<0.05). Climatic factors can affect positively or negatively the content of total dietary fibre and β-glucan, impacting on the final quality of the barley grain. Substantial variation in the content of fibre components was observed for barley cultivars grown in different years on the same location. These results indicate that environmental and genetic factors are involved in the total β-glucan content and dietary fibre of barley.

Key Words: Barley Cultivars, Genetic Factors, Bioactive Compounds.
INTRODUCTION

Natural products containing bioactive compounds, able to function in metabolism and human physiology, promoting beneficial health effects, which may delay the establishment of chronic degenerative diseases and improve the quality and life expectancy of people, are of great interest and have been widely studied in the literature.

The link between the intake of dietary fibre (DF) and various health benefits has been demonstrated in recent publications surrounding the growing incidence of various chronic diseases and obesity. In addition to the nutritional value, some components of cereals grain, as fibre and other bioactive constituents, are known to decrease the risk of chronic degenerative diseases (Önning, 2007; Slavin, 2007).

Barley is a cereal that currently has been the focus of research due to the functional importance of its nutritional components (Izydorczyk & Dexter, 2008). The interest in barley as a food grain has been reviving due to the presence of barley constituents known to prevent and to alleviate certain diseases. Barley grain is an excellent source of soluble and insoluble dietary fibre and other bioactive constituents, such as vitamin E (including toco-trienols), B-complex vitamins, minerals, and phenolic compounds (Madhujith et al., 2006; Slavin et al., 2000).

Starch, dietary fibre and protein are the main components of barley grain. Total dietary fibre (TDF) consist of insoluble and soluble fractions, both of which are resistant to digestion by the alimentary enzymes of humans. The insoluble fraction in cereal grain contains a large proportion of cellulose and has beneficial effects in the gastrointestinal tract (Jenkins et al., 1985). The soluble fractions contain mostly pectin, arabinoxylan and β-glucan.

Barley contains high levels of soluble dietary fibre, particularly mixed linkage (1,3)-(1,4)-β-D-glucans (β-glucan), which has been implicated in lowering plasma cholesterol, improving lipid metabolism, reducing glycaemic index (Behall et al., 2004; Cavallero et al., 2002; McIntosh et al., 1991), and even reducing cancer risk (Jacobs et al., 1998). Thus, the high content of β-glucans in barley gives to this cereal a special place among other cereal species.

Barley is arguably the most widely adapted cereal grain species with production at higher latitudes and altitudes and farther into deserts than any other cereal crop (Baik & Ullrich, 2008). Genetic diversity provides an ample opportunity to identify and breed barley varieties for specific end-uses. Since both genotypic and environmental factors can affect barley chemical composition, the proper selection of suitable barley grain for various uses may represent a challenge for processors and end-users, which is further exacerbated by the limited number of guidelines on the topic (Aman & Newman, 1986; Yalçin et al., 2007). Thus,
this study explores the relationship between barley bioactive properties and their genotypic and grown environmental factors.

OBJECTIVE

The main goal of this study was to determine the effects of environments factors, specifically, air temperature, insolation degree and rainfall amount, on β-glucan and dietary fibre (DF) contents in whole grain of different barley cultivars grown in Brazil.

Our results highlight that these weather variables have important effect on final barley properties.

The knowledge of the relationship between weather variables and β-glucan and dietary fibre (DF) contents of barley can contribute for the production design of barley cultivars rich in such compounds, bringing benefits for both barley processors and end consumers.

METHODS

Materials

Barley cultivars, designated BRS 225, BRS 195 and MN 743, crop year 2008 and 2009, with three replications in field trial coordinated by the Research Center of the Brazilian Agricultural Research Corporation (Embrapa Trigo / Passo Fundo) were used in this research. Whole grains of barley were grown in experimental fields in two different locations (Victor Graeff and Passo Fundo cities) in southern Brazil. The experiments which led the seed samples were sown in the first half of June. The crop was harvested in the last week of October in Victor Graeff and second week of November in Passo Fundo, in the respective years.

In order to evaluate the effects of weather variables on β-glucan and dietary fibre (DF) contents in barley, meteorological data regarding air temperature, insolation degree and rainfall amount from cultivation areas were obtained from the National Institute of Meteorology, (2012), during cultivars growing period.

Average values of air temperature, which was measured at 12 a.m., rainfall and insolation, observed during the planting and harvesting of barley in each municipality (National Institute of Meteorology, 2012), were used in order to perform statistical analysis.

Tests on barley samples

The barley samples were analyzed for moisture and protein (N x 6.25) by using AACC Methods Nos. 44-01, 46-12 and 55-10, respectively (American Association of Cereal Chemists, 1990).
For the β-glucan analysis with an enzymic method, Megazyme β-glucan and Glucose Assay Kits were used (Megazyme International, Ireland Ltd.). β-Glucan contents were assessed using the McCleary Enzymic Method for barley (McCleary and Codd, 1991). The principle of the method is depolymerization of β-glucan with endo- (1,3)-(1,4)-β-D-glucan 4-glucanohydrolase (lichenase) to oligosaccharides, hydrolysis of the oligosaccharides to glucose with purified β-D-glucosidase and determination of glucose using a glucose oxidase-peroxidase method. Total dietary fibre (TDF) contents and fractions of barley samples were determined by using AACC Standard Method No. 32-07 (AACC, 1990). Duplicate samples of milled barleys were applied to sequential enzymatic digestion by using heat stable α-amylase (Novo Nordisk A/S, Bagsvaerd, Denmark), amyloglucosidase and protease (Sigma, St. Louis, MO, USA) to remove starch and protein. For TDF, enzyme digestate was treated with ethyl alcohol to precipitate soluble dietary fibre (SDF) before filtering, and the TDF residue was washed with ethyl alcohol and acetone, and then dried and weighed. TDF residue values were corrected for protein, ash and blank. The content of soluble dietary fibre (SDF) was determined by observing the difference between TDF and IDF.

**Statistical analysis**

The average values of temperature compensated, rainfall and insolation were compared with values obtained from total dietary fibre and fractions.

The data were statistically evaluated by the one-way analysis of variance procedure using the System for Analysis and Separation Averages in Agricultural Experiment (SASM-Agri - version 4) and differences between means were identified using Scott-Knott’s test (p<0,05) (Canteri et al., 2001). When significant differences were found, the Least Significant Difference (LSD) test was used to determine the differences among mean values.

**RESULTS**

The results of β-glucan, total dietary fibre (TDF) contents and fractions of the whole barley grains grown in Passo Fundo (PF) and Victor Graeff (VG) cities are presented in Tables 1 and 2.
Table 1. β-Glucan and total dietary fibre contents of barley samples grown in different crop years in Passo Fundo city.

<table>
<thead>
<tr>
<th>Location</th>
<th>Barley lines (L)</th>
<th>β-Glucan (%) (^a)</th>
<th>Total dietary fibre (%) (^a)</th>
<th>Soluble fibre (%) (^a)</th>
<th>Insoluble fibre (%) (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BRS 195</td>
<td>2.64 (^a)</td>
<td>24.91 (^b)</td>
<td>7.03 (^a)</td>
<td>17.87 (^a)</td>
</tr>
<tr>
<td></td>
<td>BRS 225</td>
<td>2.74 (^a)</td>
<td>27.42 (^b)</td>
<td>4.82 (^c)</td>
<td>22.60 (^a)</td>
</tr>
<tr>
<td></td>
<td>MN 743</td>
<td>2.76 (^a)</td>
<td>25.47 (^b)</td>
<td>5.83 (^b)</td>
<td>19.64 (^b)</td>
</tr>
<tr>
<td></td>
<td>Mean value</td>
<td>2.71</td>
<td>25.93</td>
<td>5.89</td>
<td>20.04</td>
</tr>
<tr>
<td></td>
<td>Std. dev.</td>
<td>0.06</td>
<td>1.32</td>
<td>1.11</td>
<td>2.39</td>
</tr>
<tr>
<td>2009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BRS 195</td>
<td>1.30 (^b)</td>
<td>22.72 (^b)</td>
<td>4.25 (^d)</td>
<td>18.47 (^b)</td>
</tr>
<tr>
<td></td>
<td>BRS 225</td>
<td>1.43 (^b)</td>
<td>26.37 (^a)</td>
<td>3.73 (^d)</td>
<td>22.64 (^a)</td>
</tr>
<tr>
<td></td>
<td>MN 743</td>
<td>1.72 (^b)</td>
<td>28.85 (^a)</td>
<td>5.73 (^b)</td>
<td>23.12 (^a)</td>
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<tr>
<td></td>
<td>Mean value</td>
<td>1.48</td>
<td>25.98</td>
<td>4.57</td>
<td>21.41</td>
</tr>
<tr>
<td></td>
<td>Std. dev.</td>
<td>0.22</td>
<td>3.08</td>
<td>1.04</td>
<td>2.56</td>
</tr>
</tbody>
</table>

Means with the same letter within a column are not significantly different (p < 0.05) by least significant differences (LSD) analysis.

\(^a\) Dry weight basis.

Table 2. β-Glucan and total dietary fibre contents of barley samples grown in different crop years in Victor Graeff city.

<table>
<thead>
<tr>
<th>Location</th>
<th>Barley lines (L)</th>
<th>β-Glucan (%) (^a)</th>
<th>Total dietary fibre (%) (^a)</th>
<th>Soluble fibre (%) (^a)</th>
<th>Insoluble fibre (%) (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BRS 195</td>
<td>1.65 (^d)</td>
<td>24.05 (^a)</td>
<td>7.13 (^a)</td>
<td>16.92 (^b)</td>
</tr>
<tr>
<td></td>
<td>BRS 225</td>
<td>2.61 (^b)</td>
<td>23.70 (^a)</td>
<td>6.36 (^b)</td>
<td>17.34 (^b)</td>
</tr>
<tr>
<td></td>
<td>MN 743</td>
<td>3.31 (^a)</td>
<td>25.52 (^a)</td>
<td>4.05 (^d)</td>
<td>21.47 (^a)</td>
</tr>
<tr>
<td></td>
<td>Mean value</td>
<td>2.52</td>
<td>24.42</td>
<td>5.85</td>
<td>18.58</td>
</tr>
<tr>
<td></td>
<td>Std. dev.</td>
<td>0.83</td>
<td>0.97</td>
<td>1.60</td>
<td>2.51</td>
</tr>
<tr>
<td>2009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BRS 195</td>
<td>1.64 (^d)</td>
<td>25.13 (^a)</td>
<td>7.50 (^a)</td>
<td>17.64 (^b)</td>
</tr>
<tr>
<td></td>
<td>BRS 225</td>
<td>1.42 (^e)</td>
<td>25.63 (^a)</td>
<td>4.54 (^d)</td>
<td>21.10 (^a)</td>
</tr>
<tr>
<td></td>
<td>MN 743</td>
<td>1.82 (^c)</td>
<td>22.68 (^a)</td>
<td>5.34 (^e)</td>
<td>17.33 (^b)</td>
</tr>
<tr>
<td></td>
<td>Mean value</td>
<td>1.63</td>
<td>24.48</td>
<td>5.79</td>
<td>18.69</td>
</tr>
<tr>
<td></td>
<td>Std. dev.</td>
<td>0.20</td>
<td>1.58</td>
<td>1.53</td>
<td>2.09</td>
</tr>
</tbody>
</table>

Means with the same letter within a column are not significantly different (p < 0.05) by least significant differences (LSD) analysis.

\(^a\) Dry weight basis.
The Total dietary fibre (TDF) values statistically significant were detected only in PF, with the highest values associated to the cultivars MN 743 (2009), BRS 225 (2009) and BRS 225 (2008), while the barley lines BRS 195 (2009), BRS 195 (2008) and MN 743 (2008) had the lowest TDF values (Table 1). No statistically significant difference was observed among cultivars analyzed in VG.

The highest soluble dietary fibre (SDF) contents in PF city was detected at cultivar BRS 195 (2008), whereas the lowest values at BRS 225 (2009) and BRS 195 (2009) (Table 1). Moreover, the highest values statistically significant in VG were observed at cultivar BRS 195, crop year 2008 and 2009, while the lowest values were observed to the samples BRS 225 (2009) and MN 743 (2008) (Table 2).

Regarding the insoluble dietary fibre (IDF) the highest contents in PF were detected at cultivars MN 743 (2009), BRS 225 (2009) and BRS 225 (2008), while in VG the highest values statistically significant were observed to the samples MN 743 (2008) and BRS 225 (2009) (Table 2).

With respect to environmental factors in our study we observed that in Passo Fundo city, the average of β-glucan content was higher in 2008 with a higher mean air temperature (Figure 1) and intense sunlight (Figure 2).

**Figure 1.** Relation between β-glucan content and mean temperature (°C) in Passo Fundo (PF) city.
In Victor Graeff the same behavior was observed among cultivars regarding the mean air temperature (Figure 3). In this region was not available insolation data and therefore it was not possible to correlate this result with the percentage of \( \beta \)-glucans.

The air temperature showed a clear effect upon \( \beta \)-glucan content. The highest values of average air temperature during cultivars growing resulted on higher \( \beta \)-glucan content among cultivars in the two areas analyzed, Passo Fundo and Victor Graeff cities. Higher sunlight amounts benefited \( \beta \)-glucan formation among barley cultivars in Passo Fundo city.
With respect to rainfall, was observed that this environmental factor influenced negatively of β-glucan content, as shown (Figures 4 and 5) in Passo Fundo and Victor Graeff, with the exception only of BRS 195 (2009) in VG which had increase results on this parameter.

**Figure 4.** Relation between β-glucan content and Rainfall (mm) in Passo Fundo (PF) city.

**Figure 5.** Relation between β-glucan content and Rainfall (mm) in Victor Graeff (VG) city.

DISCUSSION

Barley grain is notable for a high content of β-glucans that ranges from 2.5% to 11.3%. The level of β-glucans in oats (2.2–7.8%), rye (1.2–2.0%), and wheat (0.4–1.4%) may also vary substantially, but it is generally lower than in barley (Andersson et al., 2004; Izydorczyk & Dexter, 2008).
According Izydorczyk and Dexter (2008) and Andersson et al. (2004), the content of \(\beta\)-glucans in barley is influenced both genetic and environmental factors and by interaction between both. The highest values of average air temperature during cultivars growing resulted on higher \(\beta\)-glucan content among cultivars in the two areas analyzed, Passo Fundo and Victor Graeff cities. Insolation also presented a statically significant influence on \(\beta\)-glucan content, increasing the content of it. As reported by Ehrenbergerová et al. (2008), higher temperatures during grain filling can provide increase of \(\beta\)-glucan content in barley grains. Rainfall amount influenced negatively the \(\beta\)-glucan contents in our study. These results corroborate the findings of Aastrup (1979) that explains its results reporting that rain might degrade \(\beta\)-glucans, decrease \(\beta\)-glucans synthesis, modify \(\beta\)-glucans to give polymers inaccessible to the \(\beta\)-glucanase and lead to leaching of the glucose, a precursor of \(\beta\)-glucans, from the flag leaf and awns. High precipitation decreases several factors, especially viscosity, which correlates positively with the water-soluble \(\beta\) glucan content of oats (Aastrup, 1979; Saastamoinen et al., 2004).

In the oat study reported from Finland, Saastamoinen et al. (2004) found that the average \(\beta\)-glucan content was significantly lower in cold rainy weather conditions than in hot dry conditions. Fastnaught et al. (1996) observed in barley higher mean glucan content and extract viscosity in 1989, which was a very hot year with low moisture. Jackson et al. (1994) found that the environmental weather factors influenced \(\beta\)-glucan content in waxy hull-less barley grain more than nitrogen fertilization. These authors concluded that \(\beta\)-glucan yield in hull-less waxy spring barley cultivars grown in hot dry regions in central Montana (USA) was higher than that obtained in oats.

The same cultivars from PF city that presented the highest value to IDF (BRS 195 and MN 743) also showed higher contents of TDF. The TDF content maintained relationship with the content of insoluble dietary fibre (IDF), and an increase in its content was observed to the crop of 2009, which showed higher rainfall and lower insolation during the planting and harvesting of grains. The soluble fibre content in PF maintained relationship with the \(\beta\)-glucan content and an increase on its was observed in the crop of 2008, which showed higher mean temperature and insolation, and lower rainfall during the planting and harvesting of grain. The same behavior was observed in VG.

CONCLUSION

The present study indicates that both environment and genetic factors have influence on the \(\beta\)-glucans and TDF content of Brazilian whole grains of barley, confirming previous studies reported in the literature.
The study also unveiled information about β-glucan and TDF content and other quality characteristics of barley grains grown in Brazil. Moreover, it was shown how climatic factors can positively or negatively affect the content of total dietary fibre and β-glucan, interfering in the final quality of the barley grain.

Substantial variation was demonstrated in the contents of fibre components of barley cultivars grown in different years on the same location.

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