



Genotypic and growing location effect on grain β -glucan content of barley under sub-tropical climates

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Abstract

Effect of genotype and growing location on barley grain beta glucan content was studied in 25 barley genotypes grown over six different locations in India. Based upon the two years results, the genotypes BCU 554, BH 963, BHS 352 and DWR 30 were having higher content of beta glucan while the genotypes Azad, BCU2030, Bilara-2, Jagriti, K 14, K 141, Lakhan, Manjula, SLOOP SA WI 3167 and SLOOP VIC VB 9953 had lower values. The growing location has also been found to affect the beta glucan content significantly. Positive simple correlation has been found between grain beta glucan content and protein, 1000 grain weight, plump kernels and test weight.

Key words: Barley, glucan, protein, genotype, growing location

Introduction

Barley (*Hordeum vulgare* L.) is the fourth important cereal after rice, wheat and maize in terms of total production in the world. The area of barley in India is 0.7 mha with total production of 1.62 million tons and productivity of 2.4 t/ha (Anonymous, 2015) and majority of this is grown under sub-tropical plains. The area of barley decreased world over and also in India, mainly due to development of high yielding dwarf varieties of wheat, assured irrigation facilities and changes in food habits. However, the area of barley has stabilized during the last one decade as there is increasing demand of barley for malt making and increasing awareness about health benefits of consuming barley as food. Besides its use for malt making, barley is increasingly being used in making multigrain *atta* (coarse wheat/cereal grain flour), barley *Daliya*, multigrain breads and breakfast cereals like Museli. The major reason for the use of barley in food

products is because of higher content of soluble fibre beta glucans in the barley grain (Sullivan et al. 2013). In a large number of studies beta glucans have been shown to decrease the blood cholesterol level, reduce blood glucose levels, improve the colon health and reduces the glycemic index of foods (Brennan and Cleary 2005; Wood 2007; Ahmad et al. 2012). Yalcin et al (2007) stated that increased incorporation of barley into human diet is recommended, because it is healthy and inexpensive. Because of these properties, barley is gaining importance as health food cereal (Sullivan et al. 2013). In a modelling study, it has been estimated that excess mortality would be attributable to dietary habits and weight related risk factors (Springmann et al. 2016), looking at this barley can be the crop of future with respect to its health benefits and changing climates

Barley grains intended for malt making and health food making have totally different quality requirements with respect to grain beta glucan content (Hu et al. 2014). For malt making the beta glucan content should be low, as higher content results in lesser grain modification, causes problem in filtration and therefore there is lesser hot water extract recovery (Wang et al. 2004 and references there in). India and China are the potential future markets, where major increase in malt consumption is expected (http://www.business-standard.com/article/current-affairs/india-malt-output-may-grow-as-beer-demand-rises-113082201191_1.html). Since malt quality is affected by grain beta glucan content, besides several other factors, information on this parameter needs to be generated. Almost all of the malt purpose barley being grown in India is under sub-tropical climates.

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However, for food purposes the barley grain should have higher grain beta glucan content. As stated earlier most of the health benefits of barley grain are rendered by its beta glucan content. Therefore multigrain *atta*, multi grain bread and *daliya* have started becoming available in the Indian market nowadays. Several studies have also been conducted to develop barley based chapattis (unleavened Indian flat bread) (Thondre and Henry 2009) and biscuits/cookies (Panjagari et al. 2015) etc. Thondre and Henry (2009) concluded that barley beta glucan significantly reduced the Glycemic Index of chapattis (Indian flat bread), particularly at doses of 4 & 8 g per serving.

Grain beta glucan content is affected by cultivar (Wang et al. 2004) and several QTLs have been reported from different chromosomes, making it a multigenic trait. The CslF/HvCslF6 and CslH genes have been implicated in partially affecting grain beta glucan content (Cory et al. 2012; Hu et al. 2014; Doblin et al. 2009). Schreiber et al. (2014) suggested possibility of involvement of three additional members of CslF gene family. However, grain beta glucan concentration is also strongly influenced by the environmental factors. The effect of genotype, environmental factors and cultural interventions have been studied in several parts of the world and some of which include China (Zhang et al. 2001), Australia (Panter and Harasymow, 2010), Turkey (Yalcin et al. 2007), Spain (Perez-Vendrell et al. 1996) etc. No published information on such traits could be traced for barley being grown at different locations in India. Such information, however, is very important for barley breeders/molecular biologists/biotechnologists and barley industry to develop/use improved varieties for food and malt purpose and for the development of barley based health food products. Under sub-tropical climatic conditions of India, the grain filling period is very small (30-40 days) as compared to the temperate conditions, therefore any information generated on grain constituent concentrations, is presumed to be very useful for the barley improvement programme under similar environments. A generalized overview of the climatic conditions, prevailing during the barley growing period between sub-tropical and temperate climates is given below:

Parameter	Sub-tropical	Temperate
Day/night temperature	25-30°C / 2-15°C	15-25°C/Approx. 15°C
Photoperiod	9-11 hr/day	14-16 hr/day
Frosting	Common	Absent

Shekhawat et al. (1999)

This study was therefore conducted to generate information on the effect of genotype and growing location on beta glucan content of barley grown at different locations in India under sub-tropical plains.

Materials and methods

A total of 25 genotypes, including 23 hulled and 2 hullless genotypes, (details given in Table 1) were

Table 1. Details of barley genotypes used in this study

Genotype	Row type	Hulled (H) or Hullless (HL)
20 th IBON 3	2	H
AMBER (K 71)	6	H
AZAD (K 125)	6	H
BCU 2030	6	H
BCU 277	6	H
BCU 554	2	H
BH 963	2	H
BH 964	2	H
BHS 352	6	HL
BILARA-2	6	H
BK 306	2	H
DWR 30	2	H
DWRB 73	2	H
DWRUB 76	2	H
HBL 276	6	HL
ICARDA 54	2	H
JAGRITI (K 287)	6	H
K 14	6	H
K 141	6	H
K 551	6	H
LAKHAN (K 226)	6	H
MANJULA (K 329)	6	H
RD 2668	2	H
SLOOP SA WL 3167	2	H
SLOOP VIC VB 9953	2	H

H = Hulled; HL = Hullless

grown during *rabi* season 2012-13 and 2013-14 at six locations spread over two zones (*North Western Plain Zone*: Karnal, Ludhiana, Hisar; *North Eastern Plain Zone*: Faizabad, Rewa and Kanpur) falling under sub-tropical climates. Each genotype was grown in two rows of 2.5 m each as single replication and normal cultural practices of the region were followed during

the growing period. The crop was fertilized with 60 kg nitrogen, 40 kg phosphorus and 20 kg potash per hectare. Grains of each genotype were then analysed at ICAR-Indian Institute of Wheat & Barley Research, Karnal. The β -glucan content was determined through an enzymatic method using Megazyme β -glucan estimation kit (Megazyme International, Ireland) following EBC method 3.11.1 for barley (McCleary & Codd, 1991). In short the grains were grinded through Tecator Cyclotech mill to pass through 0.5 mm screen and this flour was then treated with lichenase and α glucosidase and then free glucose was estimated through a colorimetric reaction. Crude protein content was predicted using Near Infrared Reflectance machine (FOSS make) by taking approximately 200 g clean and dry grains and values are expressed as protein percent on dry weight basis. The physical parameters thousand grain weight and bold grain percentage were determined as per standard IOB procedures (1997), while hectolitre weight was estimated by an instrument developed by ICAR-IIWBR. For thousand grain weight 1000 grains were counted using Pfeuffer make automatic grain counter and their weight taken on electronic balance. Bold grain percentage was estimated through Pfeuffer make Sortimat instrument and the percentage of grains retained over 2.5 mm screen (grains > 2.5 mm in width) were considered bold; while grains passing through 2.2 mm screen were designated as thin grains. Hectolitre weight or test weight was calculated by weighing 100 cc volume in grams and then converting it to kg/hectolitre.

Statistical analysis

The data of 25 genotypes, grown at six locations for two years was analyzed using the statistical software *CropStat 7.2.3* developed by IRRI, Philippines and the mean values for each genotype, location, year alongwith LSD (least significant difference) at 5 % level ($P < 0.05$) have been presented as tables.

Results

The ANOVA table shows that there was significant effect of genotype, growing year and location on the grain beta glucan content (Table 2). The interaction between the genotype and location was non-significant showing uniform effect of different environments on different genotypes, while the effect was significant for genotype \times year interaction. For protein content, thousand grain weight and bold grains effect of genotype and location was found significant; and for test weight, effect of genotype, year and location were also found significant (Table 2).

The grain beta glucan content varied from 2.9 % to 7.1 % (Table 3). Beta glucan content was > 6.5% in four genotypes viz., BCU 554, BH 963, BHS 352 and DWR 30 with highest value in DWR 30; while it was less than 3.5% in genotypes, namely, Azad, BCU2030, Bilara-2, Jagriti, K 14, K 141, Lakhan, Manjula, SLOOP SA WI 3167 and SLOOP VIC VB 9953 with lowest value in Manjula. However, Manjula and BCU 277 may be of same lineage. Among the locations lowest mean value of 4.3% was obtained at Kanpur and highest value of 5.1% was registered by Hisar (Table 4). The mean value of beta glucan content was higher (4.8%) in the year 2012-13 as compared to 2013-14 (4.5%) (Table 5). The grain protein content was significantly affected by the genotype and growing location, however the effect of year was non-significant. The protein content values ranged from 11.8% (Bilara-2) to 14.9 % (HBL-276) on dry weight basis. The genotype BK-306 is marked with special significance with > 14.0% protein content coupled with very good bold grain percentage (90%) (Table 3). There is a weak simple positive correlation (0.15) between grain beta glucan content and protein content. Lowest protein content value was obtained at Rewa (11.9 %) in and highest at Hisar (13.7%) (Table 4).

Table 2. Sources of variation and their effect of beta glucan and other parameters

Source of variation	df	Mean squares				
		BG	PROT	TGW	BL	TW
Genotype	24	29.941***	6.560*	525.134***	4605.98***	206.804***
Year	1	4.112**	7.176	20.217	251.369	373.860***
Location	5	4.273***	16.279**	392.579***	1028.86*	406.608***
Genotype \times Location	120	0.317	1.638	27.428	191.493	9.541
Genotype \times Year	24	1.108***	2.437	53.762	191.147	16.717
Genotype \times Year \times Location	125	0.394	3.986	39.748	380.437	11.305

df = Degree of freedom; BG = Beta glucan; PROT=Protein; TGW=Thousand grain weight; BL=Bold grain percentage; TW= Test weight
Significant at level * $P < 0.5$; ** $P < 0.01$; *** $P < 0.001$

Table 3. Effect of genotype on grain beta glucan content and other parameters

Genotype	BG	PROT	TGW	BL	TW
20 th IBON 3	6.5	13.0	48.3	64.1	61.9
AMBER	3.5	12.6	33.7	38.1	53.7
AZAD	3.0	12.5	38.4	44.2	54.8
BCU 2030	3.4	12.8	40.5	69.4	55.7
BCU 277	3.5	12.5	37.0	62.7	56.6
BCU 554	7.0	13.7	49.0	70.4	61.3
BH 963	6.7	12.9	44.5	61.6	60.8
BH 964	5.9	12.7	42.6	60.5	62.1
BHS 352	6.6	13.8	31.0	13.0	69.4
BILARA-2	3.2	11.8	34.9	32.3	53.0
BK 306	5.1	14.7	51.3	90.3	58.9
DWR 30	7.1	12.4	47.7	66.2	61.0
DWRUB 73	5.8	13.0	48.0	75.3	60.6
DWRUB 76	6.2	12.2	48.9	78.5	59.0
HBL 276	5.7	14.9	27.0	12.3	67.4
ICARDA 54	5.9	13.2	50.3	77.1	59.5
JAGRITI	3.3	12.3	38.5	46.7	56.3
K 14	3.1	12.7	36.5	42.7	56.4
K 141	3.0	13.1	34.4	32.7	53.8
K 551	3.6	12.6	39.1	64.9	56.2
LAKHAN	3.0	12.4	39.9	43.4	54.2
MANJULA	2.9	12.5	41.1	48.6	55.6
RD 2668	5.8	12.2	40.2	59.6	61.9
SLOOP SA WL 3167	3.0	12.6	33.0	57.0	56.2
SLOOP VIC VB 9953	3.2	12.6	35.7	69.7	59.8
SE (N=12)	0.2	0.6	1.8	5.6	1.0
LSD (5%)	0.5	1.6	5.1	15.8	2.7

BG = Grain Beta glucan (% dwb); PROT = Crude protein (% dwb); TGW = Thousand grain weight (g); BL = Bold grain percent ($e < 2.5$ mm); TW = Test weight (kg/hl)

Significant genotypic differences were observed for thousand grain weight which varied from 27 g (HBL 276) to 51.3 g (BK 306). There was a positive simple correlation between thousand grain weight and beta glucan of 0.44. Significant effect of growing location has been recorded on the thousand grain weight with highest value obtained at Faizabad (44 g) and Karnal (43.3 g). Bold or plump grain percentage (grain size of > 2.5 mm) was significantly affected by genotype and varied from 12.3 % (HBL 276) to 90.3 % (BK 306) (Table 3). There was positive simple correlation of 0.27

between beta glucan and bold/plump grain percentage. Plump grain percentage was also significantly affected by growing locations (Table 4). The test weight values ranged from 53 kg/hl (Bilara 2) to 69.4 kg/hl (BHS 352) showing significant effect of genotype on this trait (Table 3). There was a positive simple correlation between test weight and beta glucan of 0.51. The higher values of test weight obtained for BHS 352 could be attributed to hull less character of this genotype. Both growing location and year significantly affected this trait.

Table 4. Effect of growing location on barley grain beta glucan and other parameters

Location	Beta glucan (% dwb)	Protein (% dwb)	TGW (g)	Bold grains (%)	Test wt (kg/hl)
Hisar	5.1	13.7	41.3	54.3	58.2
Karnal	4.8	12.8	43.3	62.9	64.2
Ludhiana	4.6	12.9	37.4	54.5	58.3
Faizabad	4.6	13.0	44.0	57.5	57.4
Kanpur	4.3	12.8	38.1	52.6	56.2
Rewa	4.5	11.9	38.7	49.7	57.3
LSD (5%)	0.2	0.8	2.5	7.7	1.3

Discussion

In this study genotypes have been found to contain diverse range of grain beta glucan content and genotypes with high and low glucan content identified. Further the beta glucan content got affected by the growing location as well as the year. Hang et al. (2007) studied beta glucan content in 27 barley genotypes grown in two years at three locations and stated that 49% of the variability in beta glucan concentration can be attributed to year, location, year \times location, and their interaction with genotype. The amount of (1-3, 1-4)- β -glucan accumulated in barley cell walls is an important consideration for grain end-use (Cory et al. 2012). The genotypes BCU 554, BH 963, BHS 352 and DWR 30 have been found to contain higher beta glucan content. Among the four genotypes BCU 544 can be considered better for food purpose point of view as it has higher beta glucan content coupled with higher bold grain percentage. Dickin et al. (2011) studied the effect of genotype, environment and agronomic management in UK, Germany and Syria and shown that genetic variation in beta glucan concentration varied from 3.0 to 7.0 % (dwb) and was

Table 5. Effect of growing year on barley grain beta glucan and other parameters

Location	Beta glucan (% dwb)	Protein (% dwb)	TGW (g)	Bold grains (%)	Test wt (kg/hl)
Year (2012-13)	4.5	12.7	40.7	56.2	59.7
Year (2013-14)	4.8	13.0	40.2	54.3	57.5
LSD (5%)	0.1	NS	NS	NS	0.8

affected by environmental and agronomic factors. They stated that the role of beta glucan as an assimilate buffer adds complexity to interpreting the effects of environment during grain filling. Zhang et al. (2002) studied grain beta glucan content in 164 cultivars grown in China, which varied from 2.98% to 8.62%. In this study beta glucan content ranged from 2.9 to 7.1 % and was affected by environment. The present study has been conducted under sub-tropical climates, where grain filling period is much shorter as compared to European countries, therefore this study has provided preliminary information to exploit this genotypic variability in barley improvement programmes.

The genotypes Azad, BCU2030, Bilara-2, Jagriti, K 14, K 141, Lakhan, Manjula, SLOOP SA WI 3167 and SLOOP VIC VB 9953 were found to contain very low beta glucan content (< 3.5%). For malt barley, lower beta glucan content is desirable. Among these ten genotypes identified, the genotypes BCU 2030 and SLOOP VIC VB 9953 can be considered superior to others, with respect to malting quality traits, as these two have better bold grain percentage. Paynter and Harasymow (2010) stated that in view of projected changes in future climate, maltsters are looking to reduce their water use. One of the options is to develop cultivars with lower beta glucan content so that there is more penetration of water in endosperm and better germination. Barley beta glucans have been positively correlated with the kernel hardness and negatively with kernel water uptake (Gamlath et al. 2008). Harris and Fincher (2009) stated that the beta glucan concentration varies with genotype and is influenced by environmental conditions and total concentration of (1,3; 1,4)- β -glucans in barley is most frequently within the range of 4-7%. In present study also a wide range of beta glucan concentration has been observed and identified sources can be very useful resources for food and malt barley improvement programmes in sub-tropical climates. The total concentration of grain beta glucan in barley is a quantitative trait and is controlled

by additive effects of several genetic factors (Harris and Fincher 2009). Environmental and cultural factors including growing locations, year and fertilizer treatments etc. influence the grain beta glucan concentration in barley, however, because of complexity of field environments, the factors influencing the concentration are poorly understood (Harris and Fincher 2009). In this study, three factors i.e. genotype, growing location and year has affected the grain beta glucan concentration. Increasing nitrogen application has been shown to increase grain beta glucan, while increasing irrigation reduces the beta glucan content (Guler 2003). Yalcin et al. (2007) studied effect of genotype and environment on beta glucan content in 16 hull less genotypes grown in Turkey and content was affected by environmental and genetic factors. Ehrenbergerova et al. (2008) reported that higher precipitation during flowering time and grain filling period alongwith lower temperature had negative effect on beta glucan concentration, while drier and warmer weather increased beta glucan content. In this study there was no clear cut differences between locations of north western plain zone and north eastern plain zone, so that any prominent factor like temperature, growing duration or water availability to the crop could not be identified for differences among the locations. Therefore, there is need to identify the major environmental factors affecting grain beta glucan concentration and such studies need to be conducted under controlled environmental conditions. However, this could be the first report showing the grain beta glucan content in barley grains grown at multiple locations under the sub-tropical climates of Indian plains. The study shows that grain beta glucan content is affected by both genotype and growing location in the Indian barley growing conditions. Zhang et al. (2001) have suggested that environment has a greater impact on barley beta glucan than genotype in the studies carried out in China, however in a study carried out in Australia Paynter and Harasymow (2010) have suggested bigger influence of genetic factors than environmental factors. In our study, genotype seems to be the major factor deciding grain beta glucan concentration.

A positive correlation has been obtained between grain beta glucan and protein content, thousand grain weight, bold/plump grain percentage, test weight in this study. Paynter and Harasymow (2010) in a study conducted in Australia have stated that low grain beta glucan is generally associated with lower test weight, lower plump grains and lower grain protein content.

Yalcin et al. (2007) reported significant correlation between beta glucan content and some traits like plump grain percentage and 1000 kernel weight. They also stated that beta glucan content is influenced by kernel size rather than the grain density. Hang et al. (2007) reported a positive correlation of beta glucan with protein and percent plump kernels. A negative correlation between grain beta glucan content and 1000 grain weight and thin grains has been reported by Guler (2003). These grain physical parameters can be used as an indirect parameter to select for high or low grain beta glucan content during initial stages of barley improvement if number of samples are more as grain beta glucan content estimation is a tedious and costly proposition. Further work at biochemical and molecular level is required to understand the partitioning of photosynthates/precursors between different compositional components of grain, being grown under the relatively shorter window of sub-tropical climates. The genotypes identified with higher beta glucan can serve as important sources for food and malt barley improvement programme in sub-tropical climates and are useful material for conducting basic biochemical and molecular/genetic studies in relation to grain beta glucan concentration in sub-tropical climates of Indian plains. The growing location has also been found to affect the beta glucan content significantly, however no clear cut environmental factors could be identified, which affect the beta glucan concentration under sub-tropical climates. Positive simple correlation has been found between grain beta glucan content and protein, 1000 grain weight, plump kernels, test weight.

Authors' contribution

Conceptualization of research (DK); Designing of the experiments (DK); Contribution of experimental materials (RPS, ASK); Execution of field/lab experiments and data collection (RPD, ASK, SN); Analysis of data and interpretation (DK, SN); Preparation of manuscript (DK, ASK).

Declaration

The authors declare no conflict of interest.

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