



Comparative analysis of β -glucan content in wild and cultivated barley (*Hordeum vulgare*)

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ABSTRACT

β -glucan is an important element found in cereal cell walls, which is tremendously beneficial to human health and significant in industrial produce. Although some studies have been performed to investigate the β -glucan content in cultivated barley (*Hordeum vulgare* L.), little is known about it in wild barley, particularly the difference of β -glucan content between wild and cultivated varieties. In this study, β -glucan contents in wild barley from Fertile Crescent, Central Asia, Tibet, and cultivated varieties from the representative regions worldwide were systematically investigated and compared using the mixed-linkage β -glucan assay. Results showed that the mean β -glucan content of wild accessions and cultivated varieties were 5.17 % and 3.81 %, and the Coefficient of Variation (CV %) is 24.18 % and 18.11 % respectively. When statistically analysed, there was a significant variation ($p \leq 0.001$) of β -glucan content in wild and cultivated barely samples and, wild barley showed higher β -glucan content and higher genetic variation. This study provides the elite germplasm for barley β -glucan genetic improvement programs, and also shed light to trace barley domestication in relation to grain metabolite view.

Key words: β -glucan, Cultivated barley, Wild barley

Barley (*Hordeum vulgare* L.) is the fourth largest cereal crop produced worldwide, and utilized in food industry, feed industry and malt production (Arngren *et al.* 2011). It is a nutrition dense food, with low calories and less starch than other cereals such as wheat and rice. Also, being the most extensively adapted cereal grain species, which can be cultivated in fertile lands as well as desert lands, and it is the staple food source for some nations in Asia (Himalayan) and Northern Africa (Morocco and Ethiopia) (Newman and Newman 2006). Wild barley is the progenitor of cultivated barley varieties, which offers considerable potential as a genetic resource for barley improvement. Naturally occurring wild barley populations are distributed primarily across the Fertile Crescent, Central Asia and Tibet (Harlan and Zohary 1966, Nevo 1992, Wei *et al.* 2009, Zohary and Hopf 1993).

The interest in barley is surging in the last decade, due to its high content of soluble dietary fibre; especially mixed-linked (1,3;1,4)- β -D-glucan (hereafter referred to as β -glucan) (Bhatta 1999, Bilgi and Celik 2004). β -glucan is a linear molecule of partially water-soluble polysaccharides, consisting of glucose (Johansson *et al.* 2004) linked by both β -(1 \rightarrow 3) and β -(1 \rightarrow 4)-linkages (Rimsten *et al.* 2003),

and it is a major component of endosperm cell walls and sub-aleurone layer of Gramineae (Carpita 2003, Wood *et al.* 1983). Compared with other cereals, barley and oats contain relatively higher β -glucan contents and it is the most abundant soluble dietary fiber component in both crops (Johansson *et al.* 2004). However, high β -glucan content in barley can be either advantageous or disadvantageous, depending on the industry which the barley used. Low β -glucan content is required for malting and brewing, while high level is desirable for the applications in human health and nutrition (Houston *et al.* 2014).

A positive relationship between cereal consumption with the recommended β -glucan content and reduction of the risk of chronic health problems had been proved in a number of health studies. β -glucan reduces the risks of colorectal cancer, high serum cholesterol, obesity, non-insulin-dependent diabetes and cardiovascular disease such as coronary heart disease and hypertension (Klopfenstein 1988, Keogh *et al.* 2003, Li *et al.* 2003). Also, β -glucan has immune modulating properties, increasing mineral and vitamin bio-availability, and play an important role in gut physiology and influencing spatial memory performance of children (Martinez *et al.* 1992, Thorburn *et al.* 1993, Samuel 2002, Davis *et al.* 2004).

β -glucan acts a vital technological role in processed foods, particularly in the production of cheese and ice-

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cream, as a fat substitute. As a gel-forming component, β -glucans can be used for the elaboration of products with high dietary fibre (Reed and Nagodawithana 1991). At present food industry incorporate β -glucan as an ingredient in product formulation at a commercial level (Vasanthan and Temelli 2008). Though high β -glucan content is important in food industry, it is considered to be anti-nutritive in feed formulations for monogastric animals and have undesirable effects in cereal processing applications such as malting and brewing (Barrett *et al.* 1973, Hesselman *et al.* 1982, McCleary and Glennieholmes 1985, Wang *et al.* 1992).

The main methods used to determine β -glucan are Enzymatic method, Calcofluor method and Colorimetric method. Enzymatic method is the extensively used method in research laboratories (McCleary and Codd 1991), which is also called as Approved Method 32-23 (AACC International 2000, Schmitt and Wise 2009) and in this study we used this method. The impact of genetic and environmental variations for the β -glucan content in barley have been studied and the genetic factors have been found to be the most important determinant of β -glucan content (Henry 1986, Molinacano *et al.* 1997, Zhang *et al.* 2002). The variation is not only caused by genetics and environment, but can be a result of the evaluation methodology (Zhang *et al.* 2002).

It is clear, that the knowledge of β -glucan levels in barley is a valuable attribute for consumers, and it will create an opportunity for scientists and breeders to develop new cultivars, such as nutraceutical-enriched barley. The objectives of this study were to determine and compare the β -glucan contents of wild barley and cultivated barley, from different locations of the world, for the purpose of evaluation of its difference and variation. It is expected that these findings will help to identify the suitable barley genotypes and their locations to be used in breeding programs, to obtain barley with required β -glucan contents, required for the specific purpose, such as human food, livestock feed and malt production industries.

MATERIALS AND METHODS

Seeds from 60 accessions - including 32 wild barley accessions from Fertile Crescent (Mediterranean), Central Asian and Tibet regions, and 28 cultivated barley varieties from different parts of the world were tested. All seed samples were stored in seed stores of State Key Laboratory, Northwest A and F University, China, until processing for β -glucan content.

Barley grains (45 grains per sample) were ground in a centrifugal mill and passed through a 0.5 mm sieve to obtain a consistent fine powder, after drying in an oven for 24 hr. The mill was cleaned between samples.

The enzymatic measurement of β -glucan in each barley flour sample was determined by using the commercially available kit, according to the Megazyme (1,3;1,4)- β -glucan assay, based on the Streamlined method (McCleary method) (McCleary and Codd 1991, McCleary and Glennieholmes 1985). This method has been accepted by the AOAC (Method 995.16) and the AACC (Method 32-23) (BBG01/05

2004, Johansson *et al.* 2006). For each sample, two technical replicates were performed and means were used for downstream analysis. β -Glucan contents were calculated precisely as described in the Megazyme commercial kit.

In brief, complying with the streamlined procedure of Megazyme, 80–120 mg of milled flour was mixed with 200 μ l of 50% (v/v) ethanol and 4 ml sodium phosphate buffer (20 mM, pH 6.5). This mixture was incubated with lichenase enzyme (200 μ l, 50 U/ml). Then the enzymatic reaction was stopped with 5 ml sodium acetate buffer (200 mM, pH 4.0). After centrifugation, a 100 μ l aliquot was reacted to completion with 100 μ l β -glucosidase enzyme and the amount of glucose was detected with 3 ml glucose oxidase/oxidase GOPOD developing reagent. The values of the β -glucan levels were achieved for each sample as the mean \pm standard deviation. The β -glucan content was calculated as a percentage.

Those results were used to perform the statistical analysis to determine the variation of wild and cultivated barley genotypes. Statistical analyses were conducted with SPSS 23.0 software (IBM, USA).

RESULTS AND DISCUSSION

β -glucan content of selected accessions

Wild barley is the undisputed progenitor of cultivated barley and offers considerable potential as a genetic resource for barley improvement. Naturally occurring populations are distributed primarily across the Fertile Crescent, Central Asia and Tibet. The wild progenitor is still colonizing its primary habitats in the Fertile Crescent (Harlan and Zohary 1966, Nevo 1992), Central Asia (Zohary and Hopf 1993) and Tibet (Wei *et al.* 2009). The countries with significant territory within the Fertile Crescent are Iraq, Syria, Lebanon, Jordan, Israel, Palestine, Egypt, Turkey and Iran and Central Asia include Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan and Afghanistan. It attracts attention as a source of genetic variation, which is important for improvement of barley, and since it is more tolerant of drought, grows under low soil fertility and soil salinity than other major cereals (Nevo *et al.* 2012, Zohary *et al.* 2012), it is important in the regions where climatic conditions are unsuitable for other temperate cereals (and other crops), to be grown as an economically viable crop. Therefore, we selected seeds for this experiment from Fertile Crescent, Central Asia and Tibet to determine β -glucan content of wild barley, in order to provide guidelines, and recommend wild barley accessions for breeding and genetical improvement processes, to obtain required β -glucan content in cultivated barley, and also to evaluate the variation of β -glucan content between wild barley and cultivated barley (Table 1).

Descriptive statistics and frequency distribution

The null hypothesis of the study was that there is no variation in β -glucan content between wild and cultivated barley, and alternative hypothesis was, there is a variation in β -glucan between wild and cultivated barley. According

Table 1 β -glucan content of wild and cultivated barley accessions

Wild barley			Cultivated barley		
Country	Accession ID	β -glucan % (Mean \pm SD)	Country	Accession ID	β -glucan % (Mean \pm SD)
Israel	ISR_MtG_1	5.00 \pm 0.45	USA	Steptoe	4.14 \pm 0.18
Israel	ISR_MtG_2	4.28 \pm 0.08	USA	Morex	4.43 \pm 0.11
Israel	ISR_MHL_1	4.82 \pm 0.45	Canada	Harrington	3.69 \pm 0.27
Israel	ISR_MHL_2	6.40 \pm 0.59	Canada	Chopais	3.23 \pm 0.07
Israel	ISR_TBS_1	3.34 \pm 0.64	Europe	Optic	3.43 \pm 0.09
Israel	ISR_TBS_2	3.96 \pm 0.14	Europe	Century	3.86 \pm 0.04
Palestine	PSE_IG38936	5.28 \pm 0.22	Europe	Prior	3.83 \pm 0.01
Palestine	PSE_IG39565	5.99 \pm 0.29	Australia	Clipper	3.45 \pm 0.13
Palestine	PSE_IG39630	6.72 \pm 0.00	Australia	Tallon	3.12 \pm 0.04
Jordan	JOR_KFH_1	3.53 \pm 0.07	Germany	Triumph	4.09 \pm 0.17
Jordan	JOR_KFH_2	3.63 \pm 0.65	UK	Golden Promise	2.84 \pm 0.08
Jordan	JOR_KMH_1	4.04 \pm 0.42	Korea	Pgolbori	4.41 \pm 0.05
Jordan	JOR_KMH_2	3.26 \pm 0.16	Korea	Chae-Rae-Baec	3.75 \pm 0.10
Jordan	JOR_ISH_1	4.41 \pm 0.33	Korea	Baec-Meac	3.18 \pm 0.11
Jordan	JOR_ISH_2	6.52 \pm 0.19	Norway	Noga	4.11 \pm 0.65
Lebanon	LBN_IG110833	4.86 \pm 0.84	ICARDA	Atahualpa	3.17 \pm 0.03
Lebanon	LBN_IG40184	4.62 \pm 0.23	India	Mianwali	4.53 \pm 0.35
Lebanon	LBN_IG110823	5.17 \pm 0.05	India	Multan	4.14 \pm 0.22
Syria	SYR_IG39859	5.07 \pm 0.04	Japan	SumireMochi	6.15 \pm 0.07
Syria	SYR_IG39880	4.79 \pm 0.08	Japan	Mitsukiko	4.74 \pm 0.10
Syria	SYR_IG39918	4.19 \pm 0.46	China	Yunyin Barley	4.04 \pm 0.02
Turkey	TUR_IG39838	5.94 \pm 0.52	China	Xiu	3.45 \pm 0.08
Turkey	TUR_IG39839	7.67 \pm 0.36	China	Ganpi	3.47 \pm 0.01
Turkey	TUR_IG116112	7.51 \pm 0.59	China	Shanghai	3.28 \pm 0.03
Pakistan	PAK_IG39852	4.12 \pm 0.63	Sweden	Swanneck	3.60 \pm 0.05
Pakistan	PAK_IG38693	5.78 \pm 0.72	Israel	BMC	2.68 \pm 0.04
Afghanistan	AFG_IG38660	7.43 \pm 0.26	Syria	ArabiAbiad	3.85 \pm 0.04
Tajikistan	TJK_IG131790	7.01 \pm 0.78	Syria	Tadmor	3.91 \pm 0.06
Turkmenistan	TMK_IG38821	5.77 \pm 0.24			
Tibet	TBT_TBT_1	4.73 \pm 0.04			
Tibet	TBT_TBT_2	5.51 \pm 0.11			
Tibet	TBT_TBT_3	4.24 \pm 0.08			

to the results obtained from this study, the null hypothesis was rejected.

The assumption of normality was tested for both populations for β -glucan using Shapiro-Wilk test for normality, and Skewness and Kurtosis statistics suggested that the β -glucan content is reasonably normally distributed in both populations, with Skewness of 0.468 (SE = 0.414) and Kurtosis of -0.648 (SE = 0.809) in wild barley and Skewness of 1.350 (SE = 0.441) and Kurtosis of 3.854 (SE = 0.858) in cultivated barley. The mean β -glucan contents of wild and cultivated accessions were 5.17 % (SD = 1.25, Standard Error = 0.22) and 3.81 % (SD = 0.69, Standard Error = 0.13) respectively (Table 2).

The β -glucan contents of 32 wild barley accessions ranged from 3.26% for JOR_KMH_2, to 7.67% for TUR_IG39839 and approximately 60 % of accessions fell into the range of 4.0–6.0%. β -glucan content of 28 barley cultivars from different parts of the world, ranged from 2.68% for BMC a commercial cultivar from Israel, to 6.15% for Sumire Mochi a commercial cultivar from Japan and approximately 90% of cultivars fell into the range of 3.0- 5.0 % (Table 3). The wild and cultivated barley accessions differed widely in the Coefficient of Variation (CV%), where, CV% of wild and cultivated barley varieties are 24.18 % and 18.11 % respectively, and wild barley shows higher CV% (Table 3) compared to cultivated barley. Also, the boxplot (Fig

Table 2 Descriptive statistics of β -glucan content of wild and cultivated barley accessions

Descriptive statistics	Type of barley	
	Wild	Cultivated
Mean	5.17	3.81
Std. error of mean	0.22	0.13
Std. deviation	1.25	0.69
Variance	1.55	0.47
Skewness	0.468	1.350
Std. error of skewness	0.414	0.441
Kurtosis	-0.648	3.854
Std. error of kurtosis	0.809	0.858
Range	4.41	3.47
Minimum	3.26	2.68
Maximum	7.67	6.15
CV (%)	24.18	18.11

Table 3 Frequency distribution of β -glucan content of wild and cultivated barley

Type of barley	β -glucan range	Frequency	Percentage	Cumulative (%)
Wild	3 - 4	5	15.6	15.6
	4 - 5	11	34.4	50.0
	5 - 6	9	28.1	78.1
	6 - 7	3	9.4	87.5
	7 <	4	12.5	100.0
Total		32	100.0	
Cultivated	< 3	2	7.1	7.1
	3 - 4	16	57.1	64.3
	4 - 5	9	32.1	96.4
	6 - 7	1	3.6	100.0
Total		28	100.0	

1) graphically illustrates the variation of β -glucan content between wild and cultivated barley accessions.

There was a great variation in β -glucan contents in wild and cultivated barley, both from the different areas of the world (Table 3). According to the frequency table for β -glucan content for both wild and cultivated barley accessions, majority of wild barley accessions were between the range of 4-5 % and it is 34.4% of the population, while cultivated barley accessions were between the range of 3-4% and it is 57.1% of the population (Table 3 and Fig 1).

In cultivated barley, several accessions fell into < 3% of β -glucan category, while in wild barley, no accession fell into that category. In contrast, several accessions of wild barley, fell into > 7% of β -glucan category and in cultivated barley, no accession fell into that category.

Analysis of variance of barley populations

Analysis of variance for β -glucan content of wild barley accessions and barley cultivars, within the population,

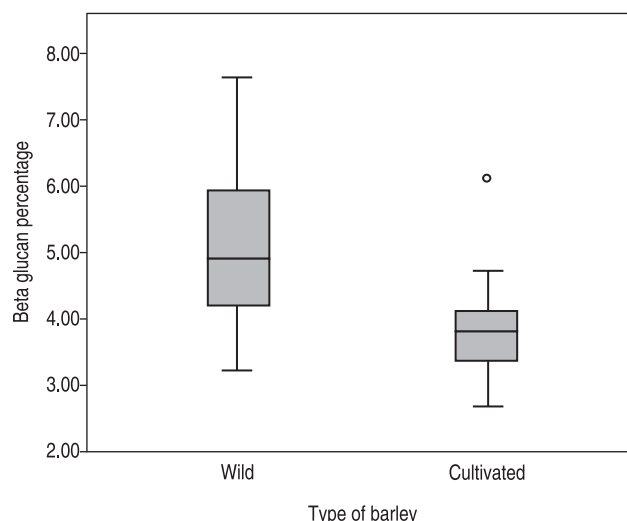


Fig 1 Variation of β -glucan content of wild and cultivated barley

showed that the effects of cultivars, locations and their interaction were highly significant ($P \leq 0.001$) (Table 4). Also, analysis of variance between wild and cultivated barley populations confirmed that there is a high significant difference ($P \leq 0.001$) in β -glucan content in wild and cultivated barley populations (Table 5).

Cluster analysis of barley populations

According to the results obtained from statistical analysis (Table 3), wild barley population can be divided into 5 groups and cultivated barley population into 4 groups, depending on the β -glucan contents. Based on groups of β -glucan, all wild and cultivated barley can be divided into 3 main categories, as low, medium and high. The low

Table 4 Analysis of variance of β -glucan content of wild and cultivated barley

Type of barley		Sum of squares	df	Mean square	F	Sig.
Wild	Between groups	96.034	31	3.098	18.059**	0.000
	Within groups	5.489	32	0.172		
Cultivated	Between groups	25.558	27	0.947	31.815**	0.000
	Within groups	0.833	28	0.030		

**Significant at 0.01 probability level

Table 5 Analysis of variance of β -glucan content between wild and cultivated barley

	Sum of squares	df	Mean square	F	Sig.
Between groups	27.972	1	27.972	26.670**	.000
Within groups	60.831	58	1.049		
Total	88.803	59			

**Significant at 0.01 probability level

category comprises β -glucan content less than 3 % and 3 - 4 %, medium 4 - 5 % and 5- 6 %, and high 6 - 7 % and above 7 %. In wild barley accessions 15.5 % fell into low, 62.5 % medium and 22.0 % high categories, where in cultivated barley, 64.0 % fell into low, 32.0 % medium and 4.0 % high, categories.

When analysing these groups, wild barley accessions AFG_IG38660, TJK_IG131790, TUR_IG116112 and TUR_IG39839, fell into one group, which is having above 7 % of β -glucan content, the highest β -glucan content group. But, in cultivated barley, the highest β -glucan group was 6 - 7 %, which consists of Sumire Mochi from Japan, which is used as human food, and no accession fell into above 7 % group. In wild barley, the lowest β -glucan group was 3 - 4 % group, which consists of JOR_KFH_1, JOR_KFH_2, JOR_KMH_2, ISR_TBS_1, and ISR_TBS_2, where in cultivated barley, the lowest group was < 3 % group, consists of Golden Promise from United Kingdom and BMC from Israel which are used for the malting purpose, whereas, there was no < 3 % group in wild barley population. It confirms results of earlier studies of β -glucan; barleys with low β -glucan content are used in the malting and brewing industries, whereas, barleys with high levels of β -glucan content are used in applications in human health and nutrition (Houston *et al.* 2014). It suggests that wild barley accessions with high β -glucan can be used for breeding programs to improve barley varieties for human food, while, low β -glucan wild barley accessions can be used to improve malting and feed varieties.

In majority of the research conducted for the β -glucan analysis, the researchers have planted original seeds in their own fields and then analysed the content of β -glucan from the harvest. In this study, we analysed β -glucan content from the original seeds directly collected from the particular location. Therefore, there is minimum chance for a change of β -glucan content due to environmental and other factors in local fields and thus, we were able to obtain actual β -glucan content of wild and cultivated accessions.

Variation of β -glucan content

The improvement of grain quality of barley depends on the collection of desirable genetic variation. The potential use of the wild barley germplasm is to be exploited largely, especially wild barley grown in Fertile Crescent, Central Asia and Tibet. These regions are recognized as the original centres of cultivated barley, where high genetic diversity was reported (Nevo 1992, Wei *et al.* 2009, Zohary and Hopf 1993). Therefore, we selected wild barley samples for this experiment from Fertile Crescent, Central Asia and Tibet, in order to provide guidelines to select suitable wild barley accessions and locations for breeding and genetical improvement processes. Genotypic and environmental variations in β -glucan content of cultivated barley have been studied extensively for several decades, while studies on wild barley is comparatively very low. In this study, we presented a quantitative analysis of health-beneficial β -glucan in wild barley and cultivated barley. The amount

of β -glucan was determined by mixed-linkage β -glucan assay procedure. Different requirements of grain quality, which depend on a diverse breeding materials, are needed to increase utilization of barley as a nutritional food, livestock feed and to improve malting quality. In present study, great variations were observed in β -glucan content in wild barley, showing its diversity, and generally β -glucan content in wild barley was higher than cultivated barley, and also it was highly significant at $P \leq 0.001$.

According to the results obtained from this study, the mean β -glucan content of wild accessions and cultivated varieties were 5.17 % (SD = 1.25, Std. Error = 0.22) and 3.81 % (SD = 0.69, Std. Error = 0.13) respectively, and the coefficient of variation (CV %) is 24.18 % and 18.11 % respectively. For 32 wild barley accessions, β -glucan content ranged from 3.26 % to 7.67 %, average for all accessions was 5.18 % and approximately 60 % of samples fell into the range 4.0 - 6.0 %. For 28 barley cultivars, β -glucan content ranged from 2.68 % to 6.15 %, average for all cultivars was 3.81 % and approximately 90% of samples fell into the range 3.0 - 5.0 %. β -glucan content of wild accessions and cultivated varieties, among and between populations, was found highly significant at $P \leq 0.001$.

Genetic background

This variability of β -glucan content is due to a wide variability in genetics of accessions and the variation in environment. Another hypothetical explanation for this variability could be, that the synthesis of β -glucan in diploid species is promoted by the function of many genes (Houston *et al.* 2014). According to studies conducted to determine environmental effect on β -glucan content (Zhang *et al.* 2002), the effects of barley cultivars, environments (locations) and their interactions on β -glucan content variation is all highly significant. Also, the genetic and environmental variations of the β -glucan content in barley have been reported in several studies (Aastrup 1979, Henry 1986, Molinacano 1997) and the genetic factors have been found to be the most important factor which determine the β -glucan content in barley (Henry 1986, Molinacano 1997, Zhang *et al.* 2002). β -glucans are synthesized by individuals of the CslF and CslH gene families (Burton *et al.* 2006, Doblin *et al.* 2009). The CslF gene family consists of ten associates (Schreiber *et al.* 2014) and is a part of the Cellulose Synthase gene superfamily, which is responsible for the production of several plant cell wall polysaccharides (Richmond and Somerville 2000). Variants among individuals of the CslF and CslH gene families and/or the genes that regulate them (directly or indirectly) control the relative abundance and specific structure of β -glucans in both the grain and the rest of the plant (Burton *et al.* 2011). Highly significant interaction between cultivars and environment implies that it is important to plant the appropriate cultivar in a particular area in order to obtain barley with required β -glucan content for the particular requirement.

In the malting and brewing industries, a low β -glucan

level is required, but in human health and nutrition, high levels of β -glucans are required (Houston *et al.* 2014). The barley accessions with higher levels of β -glucan observed in this study, can be used in breeding programs, as donors of health beneficial compound particularly in food industry, as well as potential raw material for preparation of functional foods, while, the accessions with lower levels of β -glucan can be used in livestock feed, malting and brewing industries. Also, this research highlights the need of the similar kind of further research with more different accession, to obtain the most suitable accessions or cultivars in breeding programs. In addition to chemical analysis, marker assisted selection too, can be used to obtain better results in breeding programs.

Conclusions

In this study, we systematically investigated and compared the β -glucan contents in wild and cultivated barley varieties and found that, there was a great variation of β -glucan content in wild and cultivated barley, and generally β -glucan content in wild barley was higher. By combining wild accessions with cultivated varieties through genetical improvement programs, barley varieties with required β -glucan content can be generated for the required purpose. The accessions which contained higher levels of β -glucan can be used as donors in breeding programs to obtain high β -glucan varieties to produce health beneficial food, and accessions identified with lower levels of β -glucan can be used in feed and malting purposes.

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