Journal of Cereal Research
Volume 18 (2): 383-386
Homepage: http://epubs.icar.org.in/ejournal/index.php/JWR
Short Communication

Influence of Grain Size Fractions on β-Glucan Content and β-Amylase Activity in Barley (*Hordeum vulgare* L.)

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Article history: Received: 14 May, 2026 Revised: 13 Jul., 2026 Accepted: 23 Jul., 2026

Citation: Kumar D, S Narwal OP Gupta, A Kumar and RPS Verma. 2026. Influence of Grain Size Fractions on β-Glucan Content and β-Amylase Activity in Barley (*Hordeum vulgare* L.). *Journal of Cereal Research* ***18 (2):*** *383-386.* http://doi.org/10.25174/2582-2675/2026/179003

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Barley (*Hordeum vulgare* L.) is one of the most important cereal crops used for malting and brewing industries worldwide, where grain quality plays a decisive role in determining malting performance, extract yield, wort characteristics, and brewing efficiency (Briggs, 1998; Bamforth, 2009). Among the various quality determinants, grain size, β-glucan content, and β-amylase activity are particularly important because they influence endosperm modification, wort viscosity, fermentability, and final product quality (Stewart *et al.,* 2013; Evans *et al.,* 2005).

Grain size is a primary physical characteristic used in commercial barley grading systems. Bold grains are generally preferred for malting because of their higher starch content, superior extract potential, and more uniform germination characteristics (Briggs, 1998; Bamforth, 2009). However, grain size also reflects differences in kernel development and biochemical composition. Larger kernels are generally characterized by more developed endosperm tissue and thicker cell walls, whereas smaller kernels frequently possess higher protein concentration and enzyme density (Fox *et al.,* 2003; Izydorczyk and Dexter, 2008).

β-Glucans are major non-starch polysaccharides located in the endosperm cell walls of barley grains. Although they contribute to grain structure and provide nutritional benefits as dietary fibre, elevated β-glucan concentrations are undesirable in malting and brewing because they increase wort viscosity, reduce filtration efficiency, and impair processing performance (Stewart *et al.,* 2013). The accumulation of β-glucan is regulated by cellulose synthase-like genes, particularly members of the *CslF* gene family, which control cell wall biosynthesis during grain development (Burton *et al.,* 2006; Houston *et al.,* 2014).

β-Amylase is one of the principal starch-hydrolysing enzymes responsible for maltose production during malting and mashing. It contributes significantly to diastatic power and wort fermentability and is influenced by genotype, grain protein concentration, and kernel morphology (Wang *et al.,* 2004; Mohammadi *et al.,* 2015). Previous studies have reported that smaller grains often exhibit relatively higher β-amylase activity owing to their greater aleurone-to-endosperm ratio and increased protein concentration (Fox *et al.,* 2003; Evans *et al.,* 2005).

Despite the importance of these traits, limited information is available regarding the distribution of β-glucan and β-amylase activity among grain-size fractions within Indian barley genotypes grown under subtropical environments. Furthermore, most studies have focused either on grain grading or biochemical quality parameters independently, whereas their integrated assessment remains limited. Understanding the relationship between grain size and these biochemical traits is important for improving breeding strategies, refining grain selection criteria, and enhancing malting quality. Therefore, the present study aimed to quantify variation in β-glucan content and β-amylase activity across different grain-size fractions of

selected barley genotypes and to assess their implications for malt quality improvement.

Six barley (*Hordeum vulgare* L.) genotypes, namely DWRUB 52, DWRB 92, DWRB 101, DWR 28, Alfa 93, and K 551, were evaluated during the rabi season of 2020–21 at the ICAR–Indian Institute of Wheat and Barley Research (IIWBR), Karnal, India. The field experiment was conducted using an augmented design under recommended agronomic practices. Mature grains were harvested, cleaned, and stored at −20°C until further analyses. Grain samples were equilibrated to laboratory conditions and graded using an EBC-approved Pfeuffer Sortimat grain grader. Four grain-size fractions were obtained: 2.8 mm, 2.5 mm, 2.2 mm, and <2.2 mm (Table 4). Each fraction was collected separately and used for biochemical analyses. β-Glucan content was estimated using the Megazyme Mixed-Linkage β-Glucan Assay Kit following the manufacturer's protocol. The method is based on enzymatic hydrolysis using lichenase and β-glucosidase followed by spectrophotometric quantification of released glucose. Results were expressed as percentage dry weight basis (dwb). β-Amylase activity was determined using the Megazyme β-Amylase Assay Kit according to the manufacturer's instructions. Enzyme activity was quantified spectrophotometrically and expressed as Betamyl Units g^{-1} flour. All analyses were conducted in replicates to ensure accuracy and reproducibility. Data were subjected to analysis of variance (ANOVA) to determine the effects of grain size fraction (GS), genotype (G), and their interaction (GS × G). Means were compared using the least significant difference (LSD) test at the 5% significance level. Pearson correlation coefficients were calculated to assess relationships among measured variables (Table 1). Statistical analyses were performed using RAISINS (R and AI Solutions for INferential Statistics) implemented in the R statistical environment (R Core Team, 2024; Hisham *et al.,* 2025).

Table 1: Treatment and Error Mean Squares (ANOVA)

SoV	DF	β-Amylase	β-Glucan
Grain Size (GS)	3	538.58**	18.14**
Genotype (G)	5	307.92**	1.15**
GSxG	15	17.6NS	0.32*
Error	48	19.26	0.16

SoV = Source of Variation, DF = Degrees of Freedom; Mean squares are provided for each character; * indicates significance at 5% level, ** indicates significance at 1% level, NS indicates non-significance.

The present study demonstrated that grain size significantly influenced both β-glucan accumulation and β-amylase activity in barley (Table 1 & Table 2). Larger grain fractions consistently exhibited higher β-glucan concentrations, whereas smaller fractions showed greater β-amylase activity. Similar relationships have been reported previously in barley and other cereals, where kernel development influences both cell-wall polysaccharide deposition and enzyme accumulation (Fox *et al.,* 2003; Bamforth, 2009). The elevated β-glucan content observed in bold grains may be associated with greater endosperm development and enhanced cell-wall formation during grain filling. Previous studies have shown that larger barley kernels contain more developed endosperm tissues and increased deposition of mixed-linkage β-glucans in cell walls (Evers *et al.,* 1999; Burton *et al.,* 2006). Besides these, genes belonging to the *CslF* family, particularly *CslF6*, also play a major role in regulating β-glucan biosynthesis during grain development (Burton *et al.,* 2006; Houston *et al.,* 2014).

In contrast, β-amylase activity increased significantly with decreasing grain size. Smaller kernels generally possess higher protein concentrations and a relatively larger aleurone proportion, which may contribute to enhanced enzyme accumulation (Fox *et al.,* 2003; Evans *et al.,* 2005). Genetic variation in β-amylase activity is also influenced by allelic differences at the *Bmy1* locus, which affect enzyme synthesis and thermostability (Erkkilä *et al.,* 1998; Mohammadi *et al.,* 2015).

The significant negative association observed between β-glucan content and β-amylase activity suggests a physiological trade-off between structural carbohydrate accumulation and enzymatic potential. Larger kernels tend to allocate greater assimilates towards endosperm development and cell-wall formation, whereas smaller kernels exhibit relatively higher enzyme concentration (Trafford *et al.,* 2013).

From a malting perspective, these findings indicate that grain size alone is not a sufficient criterion for quality assessment. Although bold grains possess greater starch reserves and extract potential, their higher β-glucan concentrations may hinder endosperm modification and increase wort viscosity during processing (Stewart *et*

Table 2: Effect of grain size on grain beta glucan content (% dwb)

Grain Size	Genotype					
	DWRUB 52	**DWRB 92**	**DWRB 101**	**DWR 28**	**Alfa 93**	**K 551**	**Mean**
2.8 mm	5.17 ± 0.36^{bc}	5.74 ± 0.41^{ab}	5.47 ± 0.23^{ab}	5.95 ± 0.69^{a}	4.74 ± 0.30^{cd}	4.76 ± 0.51^{cd}	**5.30 ± 0.60^{a}**
2.5 mm	4.03 ± 0.53^{efg}	4.46 ± 0.62^{cdef}	4.63 ± 0.36^{cde}	4.53 ± 0.22^{cdef}	4.47 ± 0.26^{def}	3.45 ± 0.10^{ghi}	**4.26 ± 0.54^{b}**
2.2 mm	3.97 ± 0.48^{efg}	3.55 ± 0.45^{gh}	3.98 ± 0.89^{efg}	4.52 ± 0.24^{cdef}	3.87 ± 0.19^{fg}	3.76 ± 0.17^{g}	**3.94 ± 0.50^{c}**
< 2.2 mm	2.82 ± 0.32^{ij}	2.36 ± 0.28^{j}	2.76 ± 0.40^{ij}	3.54 ± 0.29^{gh}	2.81 ± 0.19^{ij}	2.90 ± 0.10^{hij}	**2.87 ± 0.43^{d}**
Mean	4.00 ± 0.95^{bc}	4.03 ± 1.35^{bc}	4.21 ± 1.13^{b}	4.64 ± 0.96^{a}	3.97 ± 0.80^{bc}	3.72 ± 0.74^{c}	
LSD (5%)	Grain Size (GS) = 0.27, Genotype (G) = 0.33, GSxG= 0.66						

Table 3: Effect of grain size on grain beta amylase activity (beta amyl units/g of flour)

Grain Size	Genotype					
	DWRUB 52	**DWRB 92**	**DWRB 101**	**DWR 28**	**Alfa 93**	**K 551**	**Mean**
2.8 mm	7.77 ± 2.25	19.52 ± 1.69	8.44 ± 3.01	17.87 ± 0.60	19.26 ± 3.38	19.45 ± 2.08	**15.38 ± 5.68^{d}**
2.5 mm	9.59 ± 2.82	23.17 ± 3.10	10.34 ± 1.17	22.76 ± 2.20	24.87 ± 11.06	20.98 ± 1.89	**18.62 ± 7.66^{c}**
2.2 mm	14.28 ± 2.34	27.42 ± 1.84	20.43 ± 9.38	27.81 ± 1.87	22.93 ± 1.75	23.45 ± 2.32	**22.72 ± 5.91^{b}**
< 2.2 mm	24.43 ± 6.20	30.72 ± 3.71	23.38 ± 8.17	34.09 ± 3.62	29.26 ± 6.16	26.43 ± 0.70	**28.05 ± 5.89^{a}**
Mean	14.02 ± 7.48^{b}	25.21 ± 5.00^{a}	15.65 ± 8.62^{b}	25.63 ± 6.59^{a}	24.08 ± 6.78^{a}	22.58 ± 3.18^{a}	
LSD (5%)	Grain Size (GS) = 2.94, Genotype (G) = 3.6, GSxG= NS						

al., 2013; Gupta *et al.,* 2022). Conversely, smaller grains exhibit superior enzymatic activity, but may contribute less to extract yield. Therefore, breeding programmes should target genotypes that combine desirable grain size with lower β-glucan content and adequate β-amylase activity. Besides the genotype, environment also plays very important role in deciding the composition of barley grain with respect to malt quality (Zhou and Sun, 2022).

Table 4: Percent contribution of different grain sizes in different varieties

Variety	Grain percentage			
	2.8mm	**2.5mm**	**2.2mm**	**< 2.2mm**
DWRUB 52	26.3	25.5	19.0	8.0
DWRB 92	51.6	32.0	12.0	4.4
DWR 28	39.5	40.8	15.4	4.3
Alpha 93	37.0	36.7	20.0	6.4
K 551	28.3	33.1	28.4	10.2
DWRB 101	33.2	33.0	23.7	10.1
Average	**36.0**	**33.5**	**19.8**	7.2

The study further suggests that integrating grain grading with biochemical profiling could improve selection efficiency in malting barley improvement programmes. Such an approach would facilitate identification of genotypes with balanced structural and enzymatic attributes, thereby improving processing efficiency and malt quality under subtropical production environments. However, it is important to note that the present findings are based on a single-year study, and environmental factors such as temperature, moisture, and nutrient availability during grain development can also influence both grain size distribution and biochemical composition. Hence, further multi-year and multi-location studies are required to validate these results and establish more robust conclusions. Such investigations will help in developing stable barley genotypes and refining malting strategies suitable for diverse agro-climatic conditions.

Acknowledgement

Research was supported by the Indian Council of Agricultural Research, Department of Agricultural Research and Education, Government of India. Authors are grateful to Sh. Ram Pal, for providing excellent technical assistance.

Author Contributions

DK & RPS conceptualized the study, DK & SN executed the study, DK, SN, RPS, OPG & AK written and interpreted the study; all authors contributed to manuscript preparation.

Declaration of Competing Interest

The authors declare no conflict of interest.

Ethical Approval

Not applicable.

Use of AI Tools

AI tools were used for review of literature and language improvement.

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