Journal of Cereal Research
Volume 17 (2): 200-205
Homepage: http://epubs.icar.org.in/ejournal/index.php/JWR
Research Article

Barley β-Amylase: Effect of Genotype and Growing Location

Dinesh Kumar*, OP Gupta, Ram Pal, RPS Verma and GP Singh

ICAR-Indian Institute of Wheat and Barley Research, Karnal – 132001, India

Article history:
Received: 19 Sep., 2025
Revised: 22 Sep., 2025
Accepted: 28 Sep., 2025

Citation:
Kumar D, OP Gupta, R Pal, RPS Verma and GP Singh. 2025. Barley β-Amylase: Effect of Genotype and Growing Location. *Journal of Cereal Research* ***17 (2):*** *200-205. http://doi.org/10.25174/2582-2675/2025/171911*

****Corresponding author:***
E-mail: dkbiochem89@gmail.com

Abstract

Barley (*Hordeum vulgare*) β-amylase is a key starch-degrading enzyme with essential roles in seed germination, malting, and brewing and contributes significantly to the malt diastatic power. The Indian malt industry needs barley varieties with higher diastatic power especially for brewing with adjuncts. There is need to generate information on status of β-amylase activity under sub-tropical climates. In this article an attempt has been made to find out the effect of genotype and growing location on β-amylase activity. Both genotype and growing location significantly affected the grain and malt β-amylase activity. Among the genotypes ICARDA-8 has been found to possess significantly higher β-amylase activity and can serve as useful source for malting quality improvement from diastatic power point of view for the Indian malt barley improvement programme.

Keywords: Barley, β-amylase, Malt, Diastatic Power, sub-tropical climate

1. Introduction

Barley is one of the important industrial crop and major industrial uses of barley include malting and brewing. In India the malt industry is growing at a faster rate due to increasing urban population, changing life styles and food habits (Kumar *et al.*, 2021). However, in past few years the industry has revised their yardsticks for malt barley, especially because of use of adjuncts in brewing. The industry requires barley varieties with higher diastatic power and the enzyme β-Amylase is the major enzyme contributing to malt diastatic power (Kumar *et al.*, 2022a). β-amylase (EC 3.2.1.2) is an exo-acting glycoside hydrolase that catalyses the hydrolysis of α-1,4 glycosidic bonds in starch, releasing maltose units. In barley, β-amylase is the predominant amylolytic enzyme in malting, determining fermentable sugar availability during brewing. (Buchanan & Ransom-Hodgkins, 2019). The enzyme is encoded primarily by the *Bmy1* gene, located on chromosome 4H, with allelic variation linked to thermostability and malt quality (Ma *et al.*, 2016; Coventry *et al.*, 2003a). During malting, β-amylase hydrolyses starch reserves into fermentable sugars, particularly maltose, supporting yeast metabolism in brewing. Its activity directly correlates with extract yield, fermentability, and beer flavour profile (Bamforth, 2009).

India is one of the emerging markets for barley malt consumption for brewing and nutraceuticals and thus needs malting varieties meeting the international standards. The industry is importing barley to meet quality standards especially from European countries. However, the shorter grain filling period in sub-tropical plains of India as compared to the temperate European climates is a major challenge to get the best quality under Indian conditions. Since most of the parameters are governed by genotype, growing environment and cultural practices, but it's most important to breed malting barley genotypes with superior quality under Indian climatic conditions. It has been well documented that diastatic power (DP), malt extract, and fermentability are major attributes determining malt quality (Fox and Bettenhausen 2023). Very limited information is available with respect

to β-amylase status under sub-tropical climates and pertains to very small set of genotypes grown at single location (Bera *et al.*, 2018). This study aimed to evaluate the effects of genotype and growing environment on β-amylase activity in barley grain and malt under sub-tropical conditions, to identify potential donor genotypes for malting quality improvement.

2. Materials and Methods

A set of 10 genotypes (Table 1) were grown at six locations during the rabi season of 2021-22 in augmented block design. There were nine malt/feed barley varieties developed during last 15 years and one selection from from 10th EMBSN nursery received from ICARDA, Morocco. The selected genotype ICARDA-8 had shown promising results in preliminary investigations done at ICAR-IIWBR, Karnal during 2020-21. The crop was fertilized with 60 kg N (in 2 equal splits); 20 kg P and 20 kg K and all the other recommended crop management practices including weed, insect/pest control were followed as and when required. The grains were harvested at maturity and sent to ICAR-IIWBR, Karnal for malt quality analysis. Samples of 100 g of the barley variety were micro-malted in duplicate in an automated micro-malting unit (Joe White, Australia) employing a standard malting program (Kumar *et al.,* 2022b). The β-amylase activity was estimated using Megazyme kit following the method of McLeary and Codd (1989). In short grain and malt extracts were prepared by incubating 100 mg samples with 1 mL extraction buffer containing 100 mM maleic acid, 1 mM di- sodium EDTA, 0.02% sodium azide and 1 mg/ mL BSA for 2 h at room temperature. Extracts were centrifuged at 10000 g for 10 min and the supernatant retained. Enzyme activity was determined using the substrate p-nitrophenyl maltopentaoside (PNPG5). One unit of β-amylase activity is defined as the amount of enzyme required to release 1 µmol of p-nitrophenol per min in the presence of excess α-glucosidase under the defined assay conditions and is represented as Betamyl unit. For statistical analysis two-way ANOVA with genotype and location as factors was computed using the statistical software CropStat (IRRI, Philippines).

Table 1: Details of varieties used for study

S. No.	Genotype	Parentage
1	ICARDA-8	J01042/J01039 (Selection from 10th EMBSN)
2	DWRUB 52	DWR 17/K 551
3	DWRB 101	DWR 28/BH 581
4	DWRB 123	DWRUB 54/DWR 51
5	DWRB 92	DWR 28/DWR 45
6	DWRB 91	DWR 46/RD 2552
7	DWRB-160	DWRB 62/DWRB 73
8	DWRB-182	DWRUB 52/DWRB 78
9	DWRB 137	DWRB 28/DWRUB 64
10	RD2849	DWRUB 52/PL 705

3. Results and Discussion

The grain beta amylase activity varied from 13.2 units (DWRB 101) to 27.1 units (ICARDA-8). The effect of genotype was significant and among released varieties DWRB 92 has the highest activity of beta amylase (Table 2). Unlike α-amylase, which is synthesized only during germination, β-amylase is already present in the mature barley grain (Zhang *et al.,* 2014). Barley grain β-amylase is a key hydrolytic enzyme that plays a central role in starch degradation during germination and malting, releasing maltose units from the non-reducing ends of starch molecules. Its activity largely determines the diastatic power of barley, which is critical for brewing and distilling industries (Rani *et al.*, 2025; Liu *et al.*, 2025). The enzyme exists in barley grains in both bound and free forms, with genetic variation among cultivars influencing its thermostability and activity levels (Chen *et al.*, 2025). Barley varieties exhibit a broad range of β-amylase activity, reflecting both genetic and environmental influences that shape malt quality and brewing performance. Studies report that β-amylase activity can vary from as low as

40–60 U/g in some feed barleys to over 150–200 U/g in elite malting varieties, with highland and Tibetan barley landraces often showing intermediate activity levels (Wu *et al.*, 2025). A study carried out by Bera *et al.* (2018) on the grains of five malt barley varieties had reported beta amylase activity of less than 20 units.. All the genotypes taken in this study are two row types except DWRB 137, which is six rowed variety, had beta amylase activity of less than 20 units except DWRB 92 and ICARDA-8. The genotype ICARDA-8 has enzyme activity of more than 20 units al all the location indicating it as a stable character across the locations and its potential use in beta amylase activity improvement for sub-tropical climates of India. There are several reports citing genotypic differences in β-amylase activity from different parts of the world and the primary genetic determinant of this variation is the *Bmy1* locus on chromosome 4H, which encodes a cytosolic β-amylase enzyme. Different alleles of *Bmy1*–such as Sd1, Sd2L, Sd2H, Sd3, and Sd4–exhibit distinct levels of enzyme activity and thermostability, largely due to amino acid substitutions and regulatory polymorphisms (Li *et al.*, 2013; Zhang *et al.*, 2014). Comparative analyses between wild and cultivated barley from Tibet has revealed that wild barleys had higher average β-amylase activity than modern cultivars, indicating that domestication may have narrowed allelic diversity (Li *et al.,* 2014). Several novel alleles, including *Bmy1-Sd1c* and *Bmy1-Sd5*, have been identified in Chinese landraces and wild barley, featuring unique amino acid changes (e.g., A387T, C115, D165, V233, S347, V430) associated with elevated β-amylase activity (Zhang *et al.*, 2014)

Table 2: Beta Amylase activity (Betamyl Units) in grain and malt

S. No.	Genotype	Grain β-Amylase	Malt β-Amylase
1	ICARDA-8	27.1	23.3
2	DWRB 137	20.4	16.8
3	RD2849	13.8	8.4
4	DWRUB 52	13.3	4.2
5	DWRB 101	13.2	4.9
6	DWRB 123	13.3	8.7
7	DWRB 92	22.7	13.4
8	DWRB 91	19.3	11.4
9	DWRB-160	19.1	12.5
10	DWRB-182	19.8	12.5
	Location	**Grain β-Amylase**	**Malt β-Amylase**
1	Hisar	21.8	17.5
2	Pantnagar	18.9	8.9
3	Ludhiana	22.9	16.6
4	Karnal	19.6	12.4
5	Durgapura	17.9	11.1
6	Kanpur	16.9	14.3
	Genotype	**3.5***	**5.6***
LSD (5%)	**Location**	**2.1***	**3.3***
	Genotype × Location	NS	NS

Besides the role of β-amylase during grain germination and solubilization of starch during malting, the malt β-amylase activity also plays important role during the process of mashing, when residual starch is further broken down in soluble sugars. During malting, β-amylase which was largely synthesized in the developing grain and stored in an inactive form, becoming mobilized and activated upon germination (Mangan *et al.*, 2025). Its activity level significantly contributes to the diastatic power of malt, working synergistically with α-amylase

and limit dextrinase to ensure efficient starch degradation (Rani *et al.,* 2025). The enzyme exists in both free and bound forms, with the latter associated with starch granules, and their ratio varies by genotype and malting conditions (Ugarčić-Hardi *et al.*, 2003). Importantly, differences among barley varieties, malting regimes, and environmental conditions affect the final β-amylase activity in malt, influencing brewing efficiency and flavour development (Coventry *et al.*, 2003; Gebre *et al.*, 2025). Recent proteomic studies further highlight how stress conditions such as sulphur limitation alter β-amylase abundance in malt, underlining its sensitivity to both genetic and agronomic factors (Chen *et al.*, 2025). In this study, malt beta amylase activity varied from 4.2 units to 23.3 units with significant effect of genotype (Table 3). Again, the highest activity was observed in the genotype ICARDA-8, this was the only genotype to record more than 20 units among the 10 genotypes tested. There was general decrease in β-amylase activity, in all the genotypes upon malting. The possible reason can be sensitivity of enzymes to higher temperatures, as kilning temperature during malting was increased up to 80°C. Bera *et al.* (2018) also reported decrease in malt beta amylase activity after drying the malt at 50°C. The thermostability of barley β-amylase is a crucial factor influencing malt performance during brewing, as the enzyme must retain activity under the elevated temperatures of mashing. Compared to α-amylase, β-amylase is relatively heat-labile, with its activity declining significantly above 60 °C (Vinje *et al.*, 2025). Genetic variation at the *Bmy1* locus produces isoenzymes with differing thermal stabilities; some alleles confer enhanced resistance to heat inactivation, while others yield more labile enzymes that lose function quickly at brewing temperatures (Eglinton *et al.*, 1998; Wang *et al.*, 2010). Breeding programs often target thermostable β-amylase alleles to ensure consistent diastatic power and sugar release under industrial malting and mashing regimes (Li *et al.*, 2013).

Table 3: Beta Amylase activity (Betamyl Units) in grain at different locations

Genotype	Hisar	Pantnagar	Ludhiana	Karnal	Durgapura	Kanpur	Average
ICARDA-8	33.1	27.7	NA	26.5	26.7	21.8	**27.1**
DWRUB 52	10.1	10.5	28.9	8.5	10.3	11.8	**13.3**
DWRB 101	14.0	15.5	15.1	9.8	13.1	11.6	**13.2**
DWRB 123	14.3	9.9	16.3	13.0	14.2	12.3	**13.3**
DWRB 92	26.9	15.9	25.3	25.2	19.3	23.9	**22.7**
DWRB 91	22.2	14.8	24.6	19.5	19.0	15.7	**19.3**
DWRB-160	20.8	17.2	22.1	22.2	14.5	18.0	**19.1**
DWRB-182	19.5	19.2	24.6	18.2	NA	17.6	**19.8**
DWRB 137	23.9	19.2	19.7	22.8	16.3	NA	**20.4**
RD2849	14.1	19.0	15.4	9.7	10.9	NA	**13.8**
Average	**19.9**	**16.9**	**21.3**	**17.5**	**16.0**	**16.6**	

Variation in β-amylase activity among barley cultivars has also been linked to genotype–environment interactions, where higher temperatures during grain filling reduce enzyme activity, while optimal nitrogen levels enhance it (Qi *et al.*, 2006). Research comparing multiple cultivars across different climates demonstrated that environmental factors such as drought and heat stress modify β-amylase synthesis and its relationship with grain protein content (Zhang *et al.,* 2014; Wang *et al.,* 2010). Additionally, the activity of β-amylase has been found to vary with cultivar genetics and nitrogen fertilization, with distinct responses across environmental gradients (Yin *et al.*, 2002). In this study also, there was significant effect of growing location on both grain as well as malt β-amylase activity (Table 4). Higher activities were recorded in the crop grown at Ludhiana and Hisar, while lowest at Kanpur in grain and at Pantnagar in case of malt. Despite the location effect, the genotype ICARDA-8 consistently maintained higher β-amylase activity in grain as well as in malt. This genotype needs to be further studied at molecular/genetic level to dissect the reasons for higher activity in sub-tropical climates. Further, there is need to increase the β-amylase activity as well as its thermostability in the barley being grown under Indian sub-tropical climates. It may include genetic improvement through traditional as well as using molecular tools and the crop management factors affecting β-amylase activity.

Table 4: Beta Amylase activity (Betamyl Units) in malt at different location

Genotype	Hisar	Pantnagar	Ludhiana	Karnal	Durgapura	Kanpur	Average
ICARDA-8	22.4	17.8	NA	26.1	30.4	19.9	**23.3**
DWRUB 52	2.2	2.8	1.1	5.2	3.5	10.7	**4.2**
DWRB 101	6.3	2.6	5.7	4.0	2.7	7.9	**4.9**
DWRB 123	10.7	2.7	12.3	15.2	3.1	7.9	**8.7**
DWRB 92	28.6	6.4	20.5	5.7	9.2	10.1	**13.4**
DWRB 91	13.2	8.8	19.4	6.1	10.2	10.9	**11.4**
DWRB-160	14.2	10.1	16.2	10.4	6.3	17.5	**12.5**
DWRB-182	11.9	6.2	21.7	10.1	NA	12.8	**12.5**
DWRB 137	24.8	18.8	18.1	13.3	9.1	NA	**16.8**
RD2849	11.0	9.4	7.2	5.7	8.9	NA	**8.4**
Average	**14.5**	**8.6**	**13.6**	**10.2**	**9.3**	**12.2**	

Conclusion

The study indicated the significant effect of genotype and growing location on β-amylase activity in grain as well as in the malt under the sub-tropical conditions. The genotype ICARDA-8 has been identified as a potential source of higher grain and malt beta amylase activity. Future work should focus on identifying thermostable *Bmy1* alleles in ICARDA-8 background using molecular markers.

Author Contributions

DK conceptualized and planned the experiment, recorded data & prepared the manuscript; RP provided all technical assistance; RPS & GPS conceptualized, planned and edited; OPG prepared the MS and edited.

Conflict of Interest

The authors declare no conflict of interest

Ethical Approval

The article doesn't contain any study involving ethical approval.

Generative AI or AI/Assisted Technologies use in Manuscript Preparation

No

Acknowledgement

Authors are grateful to ICAR for funding the research.

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