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Genotypic and Environmental Influences on Grain, Malt and Wort Quality Traits of Barley under Subtropical Conditions: Implications for Climate-Resilient Malting Barley Breeding

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Abstract

Malting quality in barley is governed by complex genotype × environment interactions that influence grain, malt and wort characteristics. Fourteen barley (*Hordeum vulgare* L.) genotypes comprising seven indigenous and seven exotic cultivars were evaluated during two crop seasons (2020–21 and 2021–22) under subtropical conditions of north-western India to assess variability in grain, malt and wort quality traits. Significant ($P \leq 0.05$) genotypic variation was observed for test weight (57.3–66.3 kg hl^{-1}), thousand grain weight (35.2–57.1 g), bold grain percentage (38.3–92.0%), grain β-amylase activity (11.0–27.4 BAU g^{-1}) and grain β-glucan content (3.6–6.1%). The favourable 2020–21 season recorded significantly higher test weight (64.9 vs. 60.2 kg hl^{-1}), thousand grain weight (46.4 vs. 39.1 g), bold grains (78.8 vs. 51.4%) and malt β-amylase activity (16.1 vs. 7.5 BAU g^{-1}) than the warmer 2021–22 season, whereas grain protein content increased from 12.4 to 14.3%. Indigenous cultivars exhibited superior grain physical traits, with higher test weight (64.5 vs. 60.6 kg hl^{-1}), thousand grain weight (47.7 vs. 37.8 g) and bold grains (76.3 vs. 53.9%), while exotic cultivars possessed significantly higher grain β-amylase activity (22.3 vs. 15.3 BAU g^{-1}), malt α-amylase activity (224.0 vs. 157.4 CU g^{-1}), diastatic power (96.6 vs. 83.4 °Lintner), hot water extract (79.0 vs. 77.0%) and lower grain β-glucan content (4.1 vs. 5.2%). Wort β-glucan content ranged from 329.3 to 1139.1 mg L^{-1} among genotypes. DWRB 91 and DWRB 160 combined superior grain physical characteristics with adaptation to subtropical conditions, whereas Traveller and Andreia exhibited superior malting quality attributes. The study demonstrates the strong influence of seasonal environment on malting quality under subtropical conditions and identifies complementary indigenous and exotic germplasm for developing climate-resilient malting barley cultivars.

Keywords: Barley; β-glucan, genotype × environment interaction, malt enzymes, malting quality, subtropical environment

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the world's oldest cultivated cereal crops and is the principal raw material for the malting and brewing industries owing to its unique grain characteristics, including a protective husk, uniform germination, desirable starch composition and the ability to synthesize hydrolytic enzymes during malting (Briggs, 1998; Bamforth, 2003; Kumar *et al.*, 2013; Gupta

et al., 2010; Verma *et al.*, 2022). Besides its traditional use in brewing and distilling, malting barley has gained increasing importance because of the expanding demand for malt-based beverages and value-added functional food products, necessitating a consistent supply of high-quality raw material (Baik and Ullrich, 2008; Newman and Newman, 2008; Kumar *et al.*, 2022).

Malting quality is a complex trait governed by several interrelated grain, malt and wort characteristics. Grain quality attributes such as test weight, thousand grain weight, grain plumpness, protein concentration and β-glucan content influence malt modification and extract recovery, whereas malt quality is determined by enzymatic activities, including α-amylase, β-amylase, limit dextrinase and β-glucanase, together with diastatic power, friability and hot water extract. Similarly, wort quality parameters such as β-glucan concentration, free amino nitrogen and fermentable sugars affect brewing efficiency, yeast performance and the quality of the final product (Briggs, 1998; Bamforth, 2003; Fox *et al.*, 2003a,b; Kumar *et al.*, 2022). Consequently, simultaneous improvement of these quality attributes remains a major objective in malting barley breeding programmes (Psota *et al.*, 2007; Emebiri *et al.*, 2004).

The expression of malting quality traits is strongly influenced by genotype, environment and their interaction. Environmental conditions prevailing during grain filling, particularly temperature and moisture availability, affect grain development, starch deposition, protein accumulation and enzyme synthesis, ultimately determining malting performance (Gooding *et al.*, 2003; Swanston *et al.*, 1997; Fox *et al.*, 2007; Shirdelmoghanloo *et al.*, 2022). Recent studies have further emphasized that increasing climatic variability and frequent terminal heat stress can adversely affect grain physical characteristics, malt extract, starch composition and enzymatic activities, posing a significant challenge to the production of premium-quality malting barley under changing climatic conditions (Shirdelmoghanloo *et al.*, 2022). Therefore, identification of genotypes possessing stable quality traits across diverse production environments has become an important objective for barley improvement programmes (Piepho, 1998; Yan and Kang, 2003).

In the Indian subcontinent, barley is predominantly cultivated under subtropical environments where the crop frequently encounters elevated temperatures during the terminal stages of grain development. Such conditions accelerate grain filling, reduce starch accumulation and alter grain composition, thereby affecting malting quality and consistency (Kumar *et al.*, 2014; Shirdelmoghanloo *et al.*, 2022). Indian malting barley cultivars have been primarily bred for adaptation, yield stability and desirable grain quality characteristics under these environments, whereas several internationally cultivated malting varieties possess superior enzymatic properties and brewing quality (Gupta *et al.*, 2010; Verma *et al.*, 2022). Comparative evaluation of indigenous and exotic germplasm under subtropical conditions is therefore essential for identifying complementary traits that can be effectively utilized in breeding programmes aimed at developing climate-resilient malting barley cultivars (Ceccarelli, 1996; Kumar *et al.*, 2022).

Although several studies have reported the influence of genotype and environment on individual malting quality traits, comparative information on grain, malt and wort quality characteristics of indigenous and exotic barley genotypes under subtropical Indian conditions remains limited (Fox *et al.*, 2007; Psota *et al.*, 2007; Verma *et al.*, 2022). In addition, the relative contribution of seasonal environmental variation to these quality traits has not been adequately quantified under north-western plains conditions. Such information is essential for identifying stable donor genotypes and developing breeding strategies for improving malting quality under environments prone to terminal heat stress (Yan and Kang, 2003; Kumar *et al.*, 2022).

Therefore, the present study was undertaken to evaluate fourteen indigenous and exotic barley genotypes over two contrasting crop seasons under subtropical conditions of north-western India. The specific objectives were to (i) assess variability in grain, malt and wort quality traits, (ii) quantify the influence of seasonal environmental variation on malting quality, (iii) compare the performance of indigenous and exotic germplasm for important quality attributes, and (iv) identify promising parental lines for the development of climate-resilient malting barley cultivars with improved malting quality.

2. Materials and Methods

2.1 Experimental Site and Plant Material

The study was conducted at the research farm of ICAR–Indian Institute of Wheat and Barley Research (IIWBR), Karnal, Haryana, India (29.70°N, 76.99°E; 245 m above mean sea level) during two consecutive crop seasons, 2020–21 and 2021–22. The experimental site falls under the north-western plains zone of India and is characterized by a subtropical climate with cool winters and relatively warm conditions during crop maturity.

Fourteen barley (*Hordeum vulgare* L.) genotypes comprising seven indigenous and seven exotic cultivars were evaluated (Table 1). The indigenous cultivars included released Indian barley varieties possessing adaptation to subtropical production environments and specifically developed for malt purpose. The exotic cultivars represented internationally recognized malting barley varieties developed under temperate environments and widely utilized in commercial malting programmes globally. The inclusion of both indigenous and exotic germplasm enabled comparative assessment of adaptation and malting quality attributes under subtropical conditions.

The crop was grown in a randomized complete block design (RCBD) with three replications during each growing season. Each genotype was sown in mid-November and harvested during the second week of April. Recommended agronomic practices for barley cultivation were followed throughout the crop season. A fertilizer dose of 90 kg N, 20 kg P_2O_5 and 20 kg K_2O ha^{-1} was applied, with nitrogen supplied in split applications. Standard crop management practices for irrigation, weed control and plant protection were adopted as required.

Table 1. Details of Genotypes used in the study

S.N.	Genotype	Parentage/Pedigree	Suitable Season	Year of release/ registration	Country of Origin
			Indian		
1	DWRUB 52	DWR17/K551	Rabi (TS)	2007	India
2	DWRB 91	DWR46/RD2552	Rabi (LS)	2013	India
3	DWRB 101	DWR28/BH581	Rabi (TS)	2015	India
4	RD 2849	DWRUB52/PL705	Rabi (TS)	2016	India
5	DWRB 123	DWRUB54/DWR51	Rabi (TS)	2017	India
6	DWRB 160	DWRB62/DWRB73	Rabi (TS)	2020	India
7	DWRB 182	DWRUB52/DWRB78	Rabi (TS)	2020	India
			Exotic		
1	Xanadu	Viskosa × Scarlett	Spring	2006	Czech Republic
2	Andreia	DURA/TOCKA// BANTENG	Spring	2011	Argentina
3	Traveller	NA	Spring	2014	France
4	RGT Planet	Tamtam × Concerto	Spring	2015	United Kingdom
5	ABI Voyager	2B96-5038 × 2B97-4796	Spring	2011	USA
6	Explorer	Beatrix × Marnie	Spring	2012	France
7	ABI Kranti* (AbInBev)	Not Available	Not Available	Not Available	Not Available

Meteorological data including maximum temperature, minimum temperature, relative humidity and rainfall were obtained from the agrometeorological observatory located at ICAR-CSSRI. Particular emphasis was given to environmental conditions prevailing during the grain-filling period, which extended approximately from the 7th to the 14th standard meteorological week.

After harvest, grain samples were cleaned manually, packed in airtight polyethylene containers and stored at –20°C until quality analyses were performed.

2.2 Sample Preparation

Grain and malt samples were ground using a Cyclotec sample mill (Model 1093, Foss Tecator, Hillerød, Denmark) fitted with a 0.5 mm screen to obtain uniform flour. All grain, malt and wort quality analyses were carried out according to the analytical procedures recommended by the European Brewery Convention (EBC, 2010) and standard protocols routinely employed for barley quality evaluation. Unless otherwise stated, all biochemical analyses were performed in duplicate and the mean values were used for statistical analysis.

2.3 Micro-malting Procedure

Only bold grains retained on sieves ≥2.5 mm were used for malting. Micro-malting was carried out using an automated Joe White malting system (Joe White Maltings Pty Ltd., Southbank, Australia) following the procedure described by Kumar *et al.* (2022). Briefly, grain samples were subjected to controlled steeping, germination and kilning under standardized conditions. After malting, rootlets were removed manually and malt samples were stored in airtight polyethylene bags at –20°C until further analyses.

2.4 Grain Quality Traits

2.4.1. Test Weight

Test weight was determined using a hectolitre measuring apparatus developed at ICAR-IIWBR, Karnal for small grain samples. Grain weight was recorded using an electronic balance and expressed as kilograms per hectolitre (kg hl^{-1}).

2.4.2. Thousand Grain Weight

Thousand grain weight (TGW) was measured using a Contador seed counter (Pfeuffer GmbH, Germany). The weight of 1000 kernels were recorded and expressed in grams (g).

2.4.3. Grain Size Distribution

Grain grading was performed using a Sortimat laboratory grader (Pfeuffer GmbH, Germany) fitted with 2.8 mm, 2.5 mm and 2.2 mm sieves. A 100 g grain sample was sieved for three minutes. Grains retained on sieves ≥2.5 mm were classified as bold grains, whereas grains passing through the 2.2 mm sieve were classified as thin grains. Results were expressed as percentage of total grain weight.

2.4.4. Husk Content

Husk content was determined using the sodium hypochlorite digestion method as described by Kumar *et al.* (2022). Results were expressed as percentage of grain weight.

2.4.5. Grain Protein Content

Protein concentration was estimated using a Near Infrared Reflectance (NIR) grain analyser (Infratec 1241, Foss, Denmark). Protein content was expressed on a dry weight basis (%).

2.4.6. Grain β-Amylase Activity

β-Amylase activity was determined using the Betamyl assay kit (Megazyme International Ireland Ltd., Bray, Ireland) following the procedure of McCleary and Codd (1989). Enzyme activity was expressed as Beta Amylase Units per gram (BAU g^{-1}).

2.4.7. Grain β-Glucan Content

Mixed-linkage (1→3,1→4)-β-D-glucan content was estimated using a commercial assay kit (Megazyme International Ireland Ltd.) according to McCleary and Codd (1991). Results were expressed as percentage dry weight basis.

2.5 Malt Quality Traits

2.5.1. Malt β-Amylase Activity

Malt β-amylase activity was determined using the Betamyl assay procedure described by McCleary and Codd (1989). Results were expressed as BAU g^{-1} malt.

2.5.2. Malt α-Amylase Activity

α-Amylase activity was measured using the Ceralpha assay kit (Megazyme International Ireland Ltd.) following the method of McCleary and Sheehan (1987). Activity was expressed as Ceralpha Units per gram (CU g^{-1}).

2.5.3. Limit Dextrinase Activity

Limit dextrinase activity was determined using the PullG6 assay kit (Megazyme International Ireland Ltd.) according to McCleary (1992). Results were expressed as Units g^{-1} malt.

2.5.4. β-Glucanase Activity

β-Glucanase activity was estimated using the Megazyme assay procedure described by McCleary and Shameer (1987). Activity was expressed as Units kg^{-1} malt.

2.5.5. Diastatic Power

Diastatic power (DP) was determined according to the Institute of Brewing (IOB) method and expressed as degrees Lintner (°L) (Farzaneh *et al.*, 2017).

2.5.6. Malt Friability and Homogeneity

Friability was measured using a Pfeuffer friability meter (Pfeuffer GmbH, Germany) employing a 50 g malt sample. Results were expressed as percentage friability. The residue remaining after friability testing was subjected to grading in a Sortimat grader and the proportion passing through a 2.2 mm sieve was recorded as homogeneity (%).

2.5.7. Hot Water Extract

Hot water extract (HWE) was determined following EBC methodology as described by Kumar *et al.* (2022). Extract yield was expressed as percentage fine-grind dry weight basis (% fgdwb).

2.5.8. Filtration Rate

Following mashing, the mash slurry was filtered through Whatman No. 2555-1/2 filter paper. The volume of filtrate collected within one hour was recorded and expressed as mL h^{-1}.

2.6 Wort Preparation and Quality Evaluation

Malt samples were ground using a Bühler DLFU laboratory disc mill (Bühler AG, Switzerland). Mashing was performed in an automated IEC mashing bath (Australia) according to standard EBC procedures. The mash was initially incubated at 45°C followed by saccharification at 70°C for a total period of 120 min.

2.6.1. Wort β-Glucan

Wort β-glucan content was determined using the Megazyme β-glucan assay kit and expressed as mg L^{-1}.

2.6.2. Free Amino Nitrogen

Free amino nitrogen (FAN) content was estimated according to the method of Lie (1973). Absorbance was recorded at 570 nm using glycine as the standard. Results were expressed as mg L^{-1}.

2.6.3. Maltose and Glucose

Maltose and glucose concentrations were quantified using the Megazyme Maltose/Sucrose/D-Glucose assay kit (K-MASUG, Megazyme International Ireland Ltd.) and expressed as g L^{-1}.

2.7 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using CropStat software developed by the International Rice Research Institute (IRRI), Philippines. For grain and malt quality traits evaluated over two seasons, genotype, year and genotype × year interaction effects were analysed using a combined analysis of variance. Mean comparisons were performed using the least significant difference (LSD) test at the 5% probability level ($P \leq 0.05$). For wort quality traits, which were evaluated only during the 2021–22 season, one-way ANOVA was performed considering genotype as the source of variation. Results are presented as genotype means, year means and comparisons between indigenous and exotic germplasm groups wherever appropriate.

3. Results and Discussion

3.1 Environmental Conditions During Crop Growth

The two crop seasons differed markedly in meteorological conditions, particularly during the grain-filling period (7^{th}–14^{th} Standard Meteorological Weeks), which is the most critical stage determining grain composition and subsequent malting quality (Figures 1 and 2). During the 2020–21 season, relatively moderate maximum temperatures coupled with comparatively favourable relative humidity prevailed throughout grain development, providing conditions conducive to prolonged grain filling. In contrast, the 2021–22 season experienced a progressive increase in maximum temperature from approximately the 10^{th} Standard Meteorological Week onwards, accompanied by relatively lower humidity and greater fluctuations in weather conditions during the terminal stages of crop growth (Data obtained from ICAR-CSSRI, Karnal).

The contrasting environmental conditions were reflected in the quality attributes observed in the subsequent analyses. The relatively cooler grain-filling environment during 2020–21 favoured better grain development, whereas the warmer conditions during 2021–22 were associated with reduced grain size and comparatively inferior malting

quality (Tables 3 and 6). Elevated temperatures during grain filling are known to shorten the grain-filling duration, reduce starch deposition, alter protein accumulation and influence the synthesis of enzymes associated with malt quality (Gooding *et al.*, 2003; Shirdelmoghanloo *et al.*, 2022). Recent studies have further demonstrated that increasing climatic variability and terminal heat stress adversely affect kernel plumpness, malt extract and brewing quality, thereby posing a significant challenge for the production of premium malting barley under climate change scenarios (Hill *et al.*, 2024; Shirdelmoghanloo *et al.*, 2022). The distinct seasonal variation observed during the present investigation provided a suitable opportunity to evaluate the stability of grain, malt and wort quality traits under contrasting subtropical environments. Such multi-season evaluation is particularly important for identifying genotypes possessing consistent malting quality and adaptation to terminal heat stress, thereby facilitating the development of climate-resilient malting barley cultivars.

3.2 Variation in Grain Quality Traits

Considerable genotypic variation was observed for most grain quality traits, indicating substantial genetic diversity for malting quality improvement under subtropical conditions (Table 2). Test weight varied from 57.3 to 66.3 kg hl^{-1}, while thousand grain weight (TGW) ranged from 35.2 to 57.1 g. Among the genotypes, RD 2849 recorded the highest test weight (66.3 kg hl^{-1}), closely followed by DWRB 91 (66.2 kg hl^{-1}) and DWRB 101 (66.0 kg hl^{-1}), whereas RGT Planet and Explorer exhibited comparatively lower values. Similarly, DWRB 91 recorded the highest TGW (57.1 g), reflecting superior grain filling and kernel development under subtropical conditions.

Table 2: Grain quality traits of different varieties (average values of two years)

S. No.	Variety	Test Wt (kg/hl)	TGW*	Bold (%)	Thin (%)	Protein (%dwb)	GBA*	GBG*
1	Xanadu	60.1	37.7	52.9	14.8	13.3	21.4	4.2
2	Andreia	63.3	38.3	58.1	11.4	14.3	23.4	3.8
3	Traveller	60.9	37.5	55.9	12.2	13.6	27.4	4.5
4	RGT Planet	57.3	37.0	46.8	18.7	13.3	21.0	3.9
5	ABI Voyager	61.3	40.1	63.6	14.6	13.4	19.4	4.1
6	Explorer	57.8	35.2	38.3	22.0	13.5	16.1	3.6
7	ABI Kranti	63.8	38.5	61.9	10.5	13.5	27.2	4.7
8	DWRUB 52	64.4	43.1	66.3	6.8	12.5	11.2	4.9
9	DWRB 91	66.2	57.1	92.0	1.4	12.3	19.1	5.8
10	DWRB 101	66.0	45.7	74.9	3.6	13.1	11.0	4.9
11	RD 2849	66.3	43.8	67.5	6.2	12.9	11.1	4.7
12	DWRB 123	65.7	49.0	81.0	3.7	13.8	13.4	5.4
13	DWRB 160	61.1	54.0	84.6	3.1	13.0	21.5	6.1
14	DWRB 182	61.5	41.2	67.6	8.1	14.3	20.2	4.3
	LSD (5%)	3.8	5.2	19.6	9.8	NS	4.0	0.5

*TGW = Thousand grain weight (g); GBA = Grain Beta Amylase activity (beta amyl units g/flour); GBG = Grain Beta Glucan (% dwb)

Kernel plumpness is one of the most important quality attributes determining malting suitability because uniformly developed grains ensure efficient water uptake, homogeneous germination and uniform endosperm modification during malting (Fox and Bettenhausen, 2023). In the present study, bold grain percentage varied from 38.3 to 92.0%. DWRB 91, DWRB 160 and DWRB 123 produced significantly higher proportions of bold grains, whereas Explorer recorded the lowest bold grain percentage (38.3%) together with the highest proportion of thin grains (22.0%). These differences indicate substantial genetic variability for grain physical characteristics, which are known to influence malt extract recovery and processing efficiency. Similar variability among barley

genotypes has been reported under contrasting production environments (Fox *et al.*, 2003a,b; Dhall *et al.,* 2023; Kumar *et al.*, 2022; Rani *et al.*, 2024).

Grain protein content did not differ significantly among the evaluated genotypes (Table 2), indicating limited genetic variation for this trait within the germplasm evaluated. The influence of seasonal environmental conditions on grain protein concentration is discussed in the subsequent section. Nevertheless, the protein concentration of all genotypes remained within the desirable range for malting barley. Moderate grain protein is considered advantageous because excessive protein generally reduces starch content and extract yield, whereas very low protein may limit enzyme production during malting (Briggs, 1998; Bamforth, 2003).

Significant differences were also observed for grain β-amylase activity and grain β-glucan content. Grain β-amylase activity ranged from 11.0 to 27.4 BAU g^{-1}, with Traveller (27.4 BAU g^{-1}) and ABI Kranti (27.2 BAU g^{-1}) recording the highest activities, while DWRUB 52 and DWRB 101 exhibited comparatively lower values. Higher β-amylase activity is desirable because it contributes to efficient starch hydrolysis during mashing, thereby improving fermentable sugar production and brewing performance (Evans *et al.*, 2005). Recent studies have similarly reported that genetic variability in starch-degrading enzymes represents an important resource for improving malting quality through breeding (Rani *et al.*, 2024).

Grain β-glucan content varied from 3.6 to 6.1% among the genotypes. Explorer, Andreia and RGT Planet recorded comparatively lower β-glucan concentrations, whereas DWRB 160 and DWRB 91 exhibited higher values. Lower β-glucan content is preferred for malting because excessive cell-wall β-glucans increase wort viscosity, reduce filtration efficiency and adversely affect brewing performance (Gupta *et al.*, 2010). Recent investigations have highlighted the importance of β-glucan metabolism not only for processing quality, but also as a key target for developing superior malting barley cultivars through modern breeding approaches (Shirdelmoghanloo *et al.*, 2022; Kumar *et al.*, 2025).

Indigenous genotypes, particularly DWRB 91 and DWRB 160, were characterized by superior grain physical attributes, including higher thousand grain weight and bold grain percentage, reflecting their better adaptation to subtropical production environments. In contrast, the exotic genotypes Traveller, ABI Kranti and Andreia exhibited higher grain β-amylase activity and lower β-glucan content, traits that are desirable for efficient starch hydrolysis and improved brewing performance.

3.3 Effect of Growing Season on Grain Quality Traits

Seasonal environmental variation significantly influenced most grain quality traits evaluated in the present study (Table 3). The 2020–21 crop season recorded significantly higher test weight (64.9 kg hl^{-1}), thousand grain weight (46.4 g) and bold grain percentage (78.8%) compared with the 2021–22 season, which recorded corresponding values of 60.2 kg hl^{-1}, 39.1 g and 51.4%, respectively. Conversely, thin grain percentage increased significantly from 4.5% in 2020–21 to 15.1% in 2021–22, while grain protein content increased from 12.4% to 14.3%. The superior grain physical characteristics observed during 2020–21 can be attributed to the relatively favourable weather conditions prevailing during the grain-filling period (Figures 1 and 2), which promoted prolonged assimilate accumulation and improved kernel development. In contrast, the relatively higher temperatures experienced during the terminal stages of the 2021–22 crop season accelerated grain filling, resulting in smaller kernels and increased protein concentration. Similar responses have been widely reported in barley, where terminal heat stress shortens the grain-filling duration, restricts starch deposition and alters grain composition, thereby adversely affecting malting quality (Gooding *et al.*, 2003; Shirdelmoghanloo *et al.*, 2022). Recent investigations have further confirmed that increasing climatic variability and heat stress during reproductive development significantly reduce kernel plumpness and overall malting quality, highlighting the importance of developing heat-resilient barley cultivars (Hill *et al.*, 2024; Shirdelmoghanloo *et al.*, 2022).

Although grain β-amylase activity and grain β-glucan content did not differ significantly between the two growing seasons (Table 3), grain β-amylase activity showed a numerical reduction under the warmer conditions of 2021–22 (17.8 BAU g^{-1}) compared with 2020–21 (19.8 BAU g^{-1}). Likewise, grain β-glucan content remained relatively stable across seasons (4.7% and 4.6%, respectively), indicating that these biochemical traits were less influenced by seasonal variation than grain physical

characteristics. Similar observations have been reported in barley, where grain size and protein concentration generally exhibit greater environmental sensitivity than enzyme activity and β-glucan accumulation, although the latter may vary depending on genotype and stress intensity (Kumar *et al.*, 2025).

Table 3: Yearly differences in average values of grain quality traits

S. No.	Year	Test Wt	TGW	Bold	Thin	Protein	GBA	GBG
1	2020-21	64.9	46.4	78.8	4.5	12.4	19.8	4.7
2	2021-22	60.2	39.1	51.4	15.1	14.3	17.8	4.6
	LSD (5%)	1.6	2.9	7.0	3.6	0.4	NS	NS

The present findings clearly demonstrate that seasonal environmental conditions exert a pronounced influence on grain quality under subtropical environments, emphasizing the importance of multi-environment evaluation for identifying genotypes with stable malting quality. Such evaluations are essential for breeding programmes aimed at developing barley cultivars capable of maintaining desirable grain quality under increasingly variable climatic conditions.

3.4 Comparison of Indigenous and Exotic Germplasm for Grain Quality

Comparison of indigenous and exotic barley germplasm revealed distinct differences in grain quality characteristics under subtropical conditions (Table 4). Indigenous cultivars recorded significantly higher test weight (64.5 vs. 60.6 kg hl^{-1}), thousand grain weight (47.7 vs. 37.8 g) and bold grain percentage (76.3 vs. 53.9%) than exotic cultivars. Conversely, exotic germplasm exhibited a significantly higher grain β-amylase activity (22.3 vs. 15.3 BAU g^{-1}), while grain protein content did not differ significantly between the two groups.

The superior grain physical characteristics of indigenous cultivars reflect their adaptation to subtropical production environments, where selection has emphasized grain plumpness, yield stability and suitability for cultivation under terminal heat stress. Larger and bolder grains facilitate uniform water uptake during steeping and promote homogeneous malt modification, both of which are desirable attributes for efficient malting. Similar observations have been reported in Indian malting barley cultivars adapted to subtropical environments (Kumar *et al.*, 2022; Verma *et al.*, 2022).

Table 4: Differences in grain quality traits of exotic and indigenous varieties (Average values)

S. No.	Origin	Test Wt	TGW	Bold	Thin	Protein	GBA	GBG
1	Exotic	60.6	37.8	53.9	14.9	13.6	22.3	4.1
2	Indigenous	64.5	47.7	76.3	4.7	13.1	15.3	5.2
	LSD (5%)	1.7	2.5	7.8	3.7	NS	2.3	0.3

In contrast, the significantly higher grain β-amylase activity and lower β-glucan content (4.1 vs. 5.2%) observed in exotic cultivars indicate their superior biochemical potential for malting. Higher β-amylase activity enhances starch hydrolysis during mashing, whereas lower β-glucan content contributes to reduced wort viscosity and improved filtration efficiency. These characteristics are consistent with the long-term selection of European and other temperate barley cultivars for commercial malting and brewing applications (Rani *et al.*, 2024; Shirdelmoghanloo *et al.*, 2022). Recent studies have further emphasized that improving enzymatic activity while maintaining desirable grain physical characteristics remains a key objective for developing climate-resilient malting barley cultivars under diverse production environments (Kumar *et al.*, 2025).

3.5 Variation in Malt Quality Traits

Marked genotypic variation was observed for several malt quality traits (Table 5), demonstrating substantial diversity in the biochemical and modification characteristics of the evaluated germplasm. Malt β-amylase activity ranged from 6.8 to 18.7 BAU g^{-1}, with Traveller recording the highest activity, followed by RGT Planet and ABI Kranti. Malt α-amylase activity varied from 132.7 to 258.0 CU g^{-1} and was highest in Explorer, Traveller and ABI Kranti. These

Table 5: Malt quality traits of different varieties (average values of two years)

S. No.	Variety	MBA*	MAA	DP	HWE	FRIA	Homo	FR	LD	β-Glucanase
1	Xanadu	12.6	224.4	93.2	80.6	66.1	89.2	301.7	0.033	379.9
2	Andreia	14.6	222.9	94.4	80.0	67.5	90.5	252.5	0.032	454.6
3	Traveller	18.7	247.5	97.3	79.2	59.1	84.5	265.8	0.033	440.9
4	RGT Planet	15.5	215.7	95.3	79.1	68.3	92.0	274.2	0.036	297.5
5	ABI Voyager	13.4	173.0	100.8	77.3	50.7	77.3	249.2	0.037	399.4
6	Explorer	10.5	258.0	95.9	77.9	66.3	89.8	260.8	0.036	362.2
7	ABI Kranti	15.0	226.9	99.0	78.9	55.3	82.9	280.8	0.033	389.3
8	DWRUB 52	6.8	164.1	77.5	77.9	54.8	83.9	221.7	0.034	386.2
9	DWRB 91	11.2	155.5	90.6	77.9	54.2	87.0	215.8	0.031	372.6
10	DWRB 101	7.5	160.0	82.0	78.2	55.5	85.1	262.5	0.034	385.7
11	RD 2849	7.7	168.3	80.5	76.5	49.7	79.1	257.5	0.035	378.1
12	DWRB 123	8.5	132.7	77.3	76.2	43.1	72.3	245.0	0.034	361.1
13	DWRB 160	12.3	157.3	87.1	76.0	45.4	76.3	266.7	0.034	399.6
14	DWRB 182	11.0	164.1	88.8	75.9	53.1	81.6	252.5	0.040	399.0
	LSD (5%)	**7.1**	**61.1**	**NS**	**2.5**	**12.2**	**8.8**	**NS**	NS	NS

MBA = Malt Beta Amylase activity (beta amyl units/g flour); MAA = Malt Alpha Amylase activity (Ceralpha units/g flour); DP = Diastatic Power = degree Linters; HWE = Hot Water Extract (fgdwb); Fria = Malt Friability (%); Homo = Malt Homogeneity (%); FR = Wort filtration rate (ml/hr); LD = Limit dextrinase activity

differences are important because α- and β-amylases jointly determine the capacity of malt to hydrolyse starch during mashing and thereby influence fermentable sugar production (Evans *et al.*, 2005; Kumar *et al.*, 2022).

Diastatic power ranged from 77.3 to 100.8 °Lintner, with ABI Voyager, ABI Kranti and Traveller among the higher-ranking genotypes; however, the genotype effect for diastatic power was not significant at the 5% level in the two-year mean analysis. Hot water extract showed significant genotypic variation, ranging from 75.9 to 80.6% fgdwb. Xanadu and Andreia recorded the highest extract values, whereas DWRB 182, DWRB 160 and DWRB 123 were comparatively lower. Malt friability and homogeneity also differed significantly among genotypes. Friability ranged from 43.1 to 68.3%, and homogeneity from 72.3 to 92.0%, with RGT Planet, Andreia, Explorer and Xanadu generally showing better malt modification. These traits provide an indication of the extent and uniformity of endosperm modification during malting (Briggs, 1998; Kumar *et al.*, 2022).

In contrast, wort filtration rate, limit dextrinase activity and β-glucanase activity did not show significant genotypic differences in the available dataset; limit dextrinase and β-glucanase were evaluated for one season only and should therefore be interpreted cautiously. Overall, the malt-quality profile showed that several exotic cultivars combined high amylolytic enzyme activity with high extract and better malt modification. Traveller was particularly notable for β-amylase activity, Explorer for α-amylase activity, and Xanadu and Andreia for hot water extract and modification-related traits. The observed diversity provides useful parental material for combining high malting performance with the superior grain physical characteristics and subtropical adaptation present in indigenous cultivars (Rani *et al.*, 2024; Kumar *et al.*, 2022).

3.6 Effect of Growing Season on Malt Quality Traits

Growing season exerted a significant influence on most malt quality traits evaluated in the present study (Table 6). The relatively favourable environmental conditions during the 2020–21 crop season resulted in significantly higher malt β-amylase activity (16.1 vs. 7.5 BAU g^{-1}), α-amylase activity (207.3 vs. 174.2 CU g^{-1}), diastatic power (100.4 vs. 79.6 °Lintner), friability (60.5 vs. 52.2%) and wort filtration rate (278.5 vs. 236.8 mL h^{-1}) compared with the warmer 2021–22 season. Hot water extract also showed a modest but significant reduction from 78.7% in 2020–21 to 77.3% in 2021–22, whereas malt homogeneity was not significantly affected by seasonal variation.

Table 6: Yearly differences in average values of malt quality traits

S. No.	Year	MBA	MAA	DP	HWE	FRIA	Homo	FR
1	2020-21	16.1	207.3	100.4	78.7	60.5	84.3	278.5
2	2021-22	7.5	174.2	79.6	77.3	52.2	83.0	236.8
	LSD (5%)	**2.1**	**26.2**	**5.6**	**1.0**	**5.2**	**NS**	**20.4**

MBA = Malt Beta Amylase activity (beta amyl units/g flour); MAA = Malt Alpha Amylase activity (Ceralpha units/g flour); DP = Diastatic Power = degree Linters; HWE = Hot Water Extract (fgdwb); Fria = Malt Friability (%); Homo = Malt Homogeneity (%); FR = Wort filtration rate (ml/hr)

The superior malt quality observed during the 2020–21 season is closely associated with the favourable grain-filling conditions prevailing during crop growth (Figures 1 and 2). Moderate temperatures during grain development favour starch accumulation and normal endosperm development, thereby providing a suitable substrate for enzyme synthesis during malting. In contrast, elevated temperatures during grain filling accelerate physiological maturity, reduce starch deposition and adversely affect enzyme development, ultimately resulting in lower malt quality. Similar responses have been reported in barley grown under heat-stress environments, where elevated temperatures reduced malt extract, diastatic power and enzymatic activities (Gooding *et al.*, 2003; Shirdelmoghanloo *et al.*, 2022). Recent studies have likewise demonstrated that increasing climatic variability adversely affects malt quality by impairing grain filling and modifying grain biochemical composition, thereby reducing processing efficiency and brewing performance (Hill *et al.*, 2024; Shirdelmoghanloo *et al.*, 2022).

Among the malt quality parameters, β-amylase activity and diastatic power exhibited the greatest seasonal decline, indicating that starch-degrading enzymes are particularly sensitive to environmental conditions prevailing during grain development. Since diastatic power represents the collective activity of hydrolytic enzymes responsible

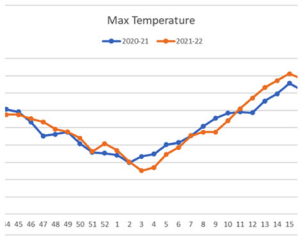

Fig. 1: Maximum temperature (°C) during different Julien weeks

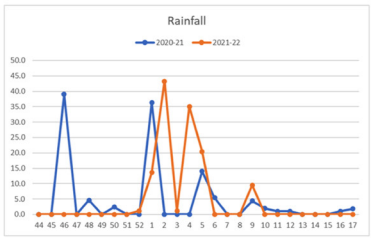

Fig. 3: Rain fall (mm) during different Julien weeks

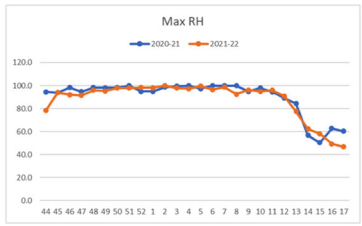

Fig. 5: Maximum relative humidity (%) during different Julien weeks

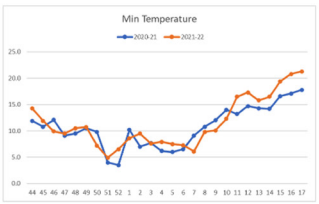

Fig. 2: Minimum temperature (°C) during different Julien weeks

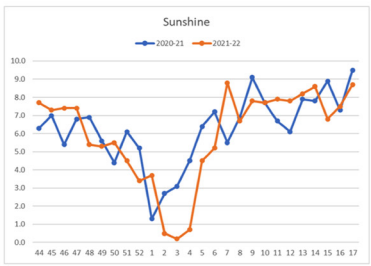

Fig. 4: Sunshine (hrs) during different Julien weeks

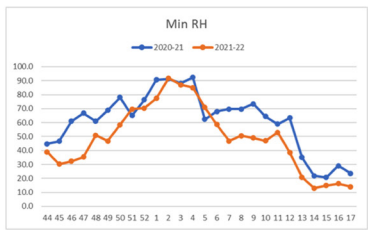

Fig. 6: Minimum relative humidity (%) during different Julien weeks

for starch degradation, its marked reduction under warmer conditions may directly affect fermentable sugar production and malt performance. Similar observations have been reported in recent studies investigating the effects of climatic stress on malting quality traits (Kumar *et al.*, 2022; Rani *et al.*, 2024).

The pronounced influence of seasonal variation on malt quality traits again highlights the importance of evaluating breeding materials across multiple environments before selection for commercial malting programmes. Genotypes exhibiting stable enzymatic activity and malt modification under contrasting seasonal conditions would provide valuable genetic resources for the development of climate-resilient malting barley cultivars adapted to subtropical production environments.

3.7 Comparison of Indigenous and Exotic Germplasm for Malt Quality

Significant differences were observed between indigenous and exotic barley germplasm for several malt quality traits (Table 7). Exotic cultivars recorded significantly higher malt β-amylase activity (14.3 vs. 9.3 BAU g^{-1}), α-amylase activity (224.0 vs. 157.4 CU g^{-1}), diastatic power (96.6 vs. 83.4 °Lintner), hot water extract (79.0 vs. 77.0%), friability (61.9 vs. 50.8%) and homogeneity (86.6 vs. 80.8%) than indigenous cultivars.

The superior enzymatic activity observed in exotic cultivars is consistent with their long history of selection for commercial malting and brewing industries, where high starch-degrading enzyme activity, efficient endosperm modification and greater extract recovery are essential quality requirements. Enhanced β-amylase and α-amylase activities contribute to increased diastatic power and improved starch hydrolysis during mashing, resulting in higher fermentable sugar production and superior brewing performance (Briggs, 1998; Kumar *et al.*, 2022). Similar observations have been reported for internationally cultivated malting barley cultivars possessing superior enzymatic potential and processing quality (Rani *et al.*, 2024; Shirdelmoghanloo *et al.*, 2022).

Although indigenous cultivars exhibited comparatively lower malt quality parameters, they demonstrated superior grain physical characteristics and better adaptation to

Table 7: Differences in malt quality traits of exotic and indigenous varieties (Average values)

S. No.	Origin	MBA	MAA	DP	HWE	FRIA	Homo	FR	LD*	β-Glucanase*
1	Exotic	14.3	224.0	96.6	79.0	61.9	86.6	269.3	0.034	389.1
2	Indigenous	9.3	157.4	83.4	77.0	50.8	80.8	246.0	0.035	383.2
	LSD (5%)	**2.6**	**22.9**	**6.6**	**1.0**	**4.9**	**3.7**	**21.8**	**NS**	**NS**

*One year data (2021-22)

MBA = Malt Beta Amylase activity (beta amyl units/g flour); MAA = Malt Alpha Amylase activity (Ceralpha units/g flour); DP = Diastatic Power = degree Linters; HWE = Hot Water Extract (fgdwb); Fria = Malt Friability (%); Homo = Malt Homogeneity (%); FR = Wort filtration rate (ml/hr)

subtropical production environments (Table 4). Therefore, their relatively lower enzymatic activity represents an opportunity for genetic improvement through strategic hybridization with elite exotic malting germplasm. Recent studies have emphasized that combining locally adapted germplasm with superior malting-quality donors is an effective strategy for developing climate-resilient barley cultivars possessing both agronomic adaptation and enhanced processing quality (Kumar *et al.*, 2025).

Limit dextrinase activity and β-glucanase activity did not differ significantly between indigenous and exotic germplasm (Table 7). However, these observations should be interpreted with caution, as both traits were evaluated during a single crop season (2021–22). Multi-season and multi-location validation will be necessary to establish the stability of these enzymatic traits under diverse environmental conditions.

3.8 Variation in Wort Quality Traits

Wort quality traits were evaluated during the 2021–22 crop season only; therefore, the results should be interpreted with appropriate caution pending validation across additional environments. Nevertheless, significant genotypic variation was observed for wort β-glucan content, whereas free amino nitrogen (FAN), maltose and glucose contents did not differ significantly among the evaluated genotypes (Table 8). Wort β-glucan concentration ranged from 329.3 to 1139.1 mg L^{-1}. Among the evaluated genotypes, Explorer recorded the lowest β-glucan concentration (329.3 mg L^{-1}), followed by RGT Planet (514.8 mg L^{-1}) and Xanadu (562.8 mg L^{-1}), whereas DWRB 160 (1139.1 mg L^{-1}), DWRB 123 (1042.8 mg L^{-1}) and DWRB 91 (989.6 mg L^{-1}) exhibited comparatively higher concentrations. Lower wort β-glucan content is desirable because excessive β-glucans increase wort viscosity, reduce filtration efficiency and may adversely affect beer clarity and processing efficiency (Bamforth, 2003). Similar associations between lower wort β-glucan concentration and improved brewing performance have been reported in recent studies on malting barley quality (Shirdelmoghanloo *et al.*, 2022; Kumar *et al.*, 2025).

Free amino nitrogen (FAN) varied from 163.9 to 198.3 mg L^{-1}, but genotypic differences were not statistically significant (Table 8). Likewise, maltose and glucose concentrations exhibited only numerical variation among the genotypes. Although these metabolites are important determinants of yeast nutrition and fermentation efficiency, their relatively narrow range suggests that genetic differences among the evaluated germplasm had limited influence on these traits under the prevailing environmental conditions. Similar observations have been

Table 8: Wort quality traits of different varieties (single year data)

S. No.	Variety	WBG	FAN	Maltose	Glucose
1	Xanadu	562.8	169.8	16.395	6.268
2	Andreia	583.5	188.3	15.007	7.242
3	Traveller	660.2	190.9	14.492	7.030
4	RGT Planet	514.8	186.7	12.136	6.543
5	ABI Voyger	749.4	172.2	9.953	7.105
6	Explorer	329.3	198.3	15.589	5.931
7	ABI Kranti	625.6	191.9	16.976	5.944
8	DWRUB 52	757.3	191.2	11.140	5.869
9	DWRB 91	989.6	179.9	11.484	6.818
10	DWRB 101	750.9	192.0	15.994	6.516
11	RD 2849	870.0	177.0	14.189	6.905
12	DWRB 123	1042.8	168.1	16.016	8.866
13	DWRB 160	1139.1	163.9	15.422	7.504
14	DWRB 182	672.9	172.1	10.784	8.453
	LSD (5%)	**303.5**	**NS**	**NS**	**NS**

WBG = Wort Beta Glucan (ppm); FAN = Free Amino Nitrogen (ppm); Maltose (g/litre); Glucose (g/litre)

reported in barley quality studies where wort carbohydrate composition and FAN were influenced more strongly by malting conditions than by genotype alone (Farzaneh *et al*., 2017).

The considerable variation observed for wort β-glucan, together with the relatively stable FAN and fermentable sugar contents, suggests that β-glucan concentration remains one of the most discriminating wort quality parameters for selection of malting barley under subtropical conditions. However, since these observations were obtained from a single crop season, further multi-environment evaluation is required to establish the stability of wort quality traits before their routine use in breeding programmes. Comparison of indigenous and exotic germplasm (Table 9) revealed that exotic cultivars produced significantly lower wort β-glucan concentrations than indigenous cultivars (575.1 vs. 888.9 mg L^{-1}), indicating superior brewing quality. However, FAN, maltose and glucose contents did not differ significantly between the two germplasm groups. These findings further support the superior processing quality of exotic malting barley while confirming that the principal distinction between the two groups lies in cell-wall β-glucan degradation rather than fermentable sugar composition.

3.9 Implications for Malting Barley Improvement Under Subtropical Conditions

The present investigation demonstrated that both genotype and seasonal environmental conditions play pivotal roles in determining grain, malt and wort quality traits of barley under subtropical production environments. Grain physical characteristics, including test weight, thousand grain weight and grain plumpness, were markedly influenced by seasonal weather conditions during grain filling, whereas grain β-amylase activity and β-glucan content exhibited comparatively greater stability across seasons. In contrast, malt quality parameters, particularly β-amylase activity, α-amylase activity, diastatic power, friability, hot water extract and filtration rate, showed greater sensitivity to seasonal environmental variation, highlighting the importance of favourable grain-filling conditions for the development of superior malting quality. Wort quality traits exhibited relatively smaller genotypic differences, although their interpretation should be made cautiously because these observations were based on a single crop season.

Comparison of indigenous and exotic germplasm revealed complementary strengths for malting barley improvement. Indigenous cultivars possessed superior grain physical

Table 9: Differences in wort quality traits of exotic and indigenous varieties (Average values)

S. No.	Origin	WBG	FAN	Maltose	Glucose
1	Exotic	575.1	185.4	14.364	6.580
2	Indigenous	888.9	177.7	13.575	7.276
	LSD (5%)	**122.8**	**NS**	**NS**	**NS**

Table 10: Summary of genotypic and environmental influences on grain, malt and wort quality traits of barley under subtropical conditions

Trait	Genotype effect	Year effect	Superior germplasm source	Breeding implication
Test weight	Significant	Significant	Indigenous	Grain physical quality
TGW	Significant	Significant	Indigenous	Kernel plumpness
Bold grains	Significant	Significant	Indigenous	Malting suitability
Grain β-amylase	Significant	NS	Exotic	Enzyme activity
Grain β-glucan	Significant	NS	Exotic	Brewing quality
Malt β-amylase	Significant	Significant	Exotic	Malting quality
α-Amylase	Significant	Significant	Exotic	Saccharification
Diastatic power	Significant	Significant	Exotic	Brewing efficiency
Hot water extract	Significant	Significant	Exotic	Extract yield
Wort β-glucan	Significant	One year	Exotic	Filtration efficiency

characteristics and better adaptation to subtropical production environments, whereas exotic cultivars consistently exhibited enhanced enzymatic activity, improved malt modification, higher extract potential and lower β-glucan content, traits that are highly desirable for commercial malting and brewing. These findings indicate that the simultaneous improvement of agronomic adaptation and malting quality cannot be achieved by selection for a single group of germplasm alone.

Among the evaluated genotypes, DWRB 91 and DWRB 160 emerged as valuable donors for superior grain physical quality and adaptation to subtropical environments, while Traveller, Andreia and ABI Kranti exhibited outstanding enzymatic and malting quality attributes. The complementary nature of these genotypes provides excellent opportunities for strategic hybridization aimed at combining superior grain development with enhanced processing quality.

Overall, the study highlights the necessity of multi-environment evaluation for reliable assessment of malting quality traits under subtropical conditions. Integrating the superior adaptation and grain physical characteristics of indigenous cultivars with the enhanced enzymatic and processing qualities of elite exotic germplasm will facilitate the development of climate-resilient malting barley cultivars capable of consistently meeting the quality requirements of the malting and brewing industries under increasingly variable climatic conditions.

4. Conclusion

The present study demonstrated that both genotype and seasonal environmental conditions exert significant influences on grain, malt and wort quality traits of barley under subtropical production environments. Grain physical characteristics, including test weight, thousand grain weight and grain plumpness, were strongly affected by seasonal variation, whereas malt quality parameters, particularly β-amylase activity, α-amylase activity, diastatic power, friability and hot water extract, exhibited pronounced responses to environmental conditions prevailing during grain filling. In contrast, grain β-amylase activity and β-glucan content showed relatively greater stability across seasons, while wort quality traits exhibited comparatively limited genotypic variation under the single-season evaluation.

The comparative assessment of indigenous and exotic germplasm revealed complementary strengths for malting barley improvement. Indigenous cultivars possessed superior grain physical characteristics and better adaptation to subtropical production environments, whereas exotic cultivars exhibited enhanced enzymatic activity, improved malt modification, higher extract potential and lower β-glucan content, making them valuable sources of malting quality traits. Among the evaluated genotypes, DWRB 91 and DWRB 160 were identified as promising donors for superior grain physical quality and adaptation, while Traveller and Andreia emerged as valuable sources of enhanced malting quality attributes. The integrated summary presented in Table 10 highlights the relative contributions of genotype and seasonal environment to the major quality traits and identifies their potential utilization in barley improvement programmes. These findings emphasize that simultaneous improvement of agronomic adaptation and malting quality requires the strategic utilization of complementary indigenous and exotic germplasm coupled with multi-environment evaluation to ensure trait stability.

Overall, the study provides valuable information for barley breeders and the malting industry by identifying promising parental resources and key quality traits for the development of climate-resilient malting barley cultivars capable of consistently meeting the quality requirements of the malting and brewing industries under subtropical production environments. The findings of the present investigation provide a useful framework for integrating grain physical characteristics and malting quality traits in future barley breeding programmes targeting subtropical production environments

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Author contributions

DK conceptualized the study; DK, SN, Rampal and RPS executed the study; DK, SN, OPG, AK and RPS analysed and interpreted the results; all authors contributed to manuscript writing and approved the final version.

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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