DIFFERENCES IN NUTRIENT FUNCTIONAL COMPOSITION AMONG DIFFERENT TYPES OF GRAINS IN BARLEY (*HORDEUM VULGARE* L.) RECOMBINANT INBRED LINES

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Abstract. This study aimed to investigate the genetic variation, environmental effects, and trait interrelationships in the nutrient and functional composition of barley (*Hordeum vulgare* L.) grains. A total of 193 recombinant inbred lines (RILs), derived from a cross between Ziguangmangluoerleng (ZGMLEL) and Schooner, along with their parental lines, were evaluated to identify promising genotypes for nutritional improvement and breeding. Field trials were conducted over three consecutive years (2021–2023) using a randomized complete block design with three replications. Analysis of variance (ANOVA) revealed highly significant ($p < 0.01$) effects of genotype and year on all studied traits, total anthocyanin (Tac), moisture, amylose, starch, β-glucan (BG), fatty acid (FA), and protein content, indicating both genetic and environmental influences. However, the genotype-by-year interaction was non-significant ($p > 0.05$) for most traits, suggesting stable genetic rankings across years. The mean values of Tac ranged from 0.14 (2022) to 1.57 (2023), with genotype z79 consistently exhibiting the highest levels (1.35 in 2021, 1.09 in 2022, and 1.57 in 2023). Starch content was relatively stable, with a maximum of 55.80% (2021), while amylose varied from 15.00% (2023) to 27.02% (2021). BG content showed significant fluctuations, with means ranging from 6.79% (2023) to 9.29% (2022). Fatty acid and protein contents also varied, with protein reaching a maximum of 23.81% in 2023. Genotypes z79, z11, z95, and z146 consistently outperformed the parental means, making them promising candidates for breeding programs aimed at enhancing nutritional quality and functional food applications. Correlation analysis highlighted key trade-offs, particularly a strong negative correlation between starch and protein (-0.90** in 2021, -0.85* in 2022 and -0.82** in

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2023), and a strong positive correlation between amylose and BG (0.34** in 2023). These findings provide valuable insights for breeding programs aimed at improving the nutritional quality of barley.

Keywords: *barley, anthocyanin, amylose, β-glucan, protein, correlation*

Introduction

Barley (*Hordeum vulgare* L.) is a cereal of great international importance, ranking number four in production. The commodity is highly flexible, with uses ranging from animal feed and malt to human intake. Apart from its classical utilization, there is a rising recognition of its potential for health-related functions, owing to its distinct content of nutritional functional components (Wijekoon et al., 2022; Graton et al., 2024). Key nutritional components in barley include β-glucans (BG), total anthocyanins (Tac), starch, proteins, and fatty acids (FA), each contributing to its functional food value and health benefits. Knowledge of the complex interrelationships of these factors is of great importance in maximizing utilization of barley for different purposes (Mohamed et al., 2010; Han et al., 2018). The main objective of this study was to dissect the genetic and environmental contributions to key grain quality traits, and to understand how these traits correlate with one another. This knowledge is essential for selecting superior genotypes in breeding programs focused on nutritional and functional improvement of barley.

The nutritional composition of the seed of barley is a multi-component system depending on environmental and genetical factors. The relative amount ratio of starch, protein, BG, and other factors determines its nutritional functional components. Anthocyanins are powerful antioxidants, which play protective roles in oxidative stress, reducing atherosclerosis diseases, cancer, and diseases of neurodegeneration (Raj et al., 2023; Liaqat et al., 2024). The genetic mechanisms of anthocyanins is varied in different cultivars of barley, and environmental factors of temperature, intensity of radiation, and provision of nutrition in soil modulate its content (Hosseinian et al., 2008).

Starch and amylose content are internal determiners of end-use in barley, particularly in brewing and in foods. The primary carbohydrate storage component of grains of barley, starch is a primary contributor of human dietary energy. The proportion of amylose in total starch contributes to texture, cook-up, and digestibility (Shvachko et al., 2021; Geng et al., 2022). Optimal starch content in selected barley lines is critical for industrial appropriateness as well as for maximizing nutritional attributes (Oluwajuyitan et al., 2021; Xiong et al., 2022). Barley is a rich source of a soluble, rich in a rich variety of valuable attributes, such as BG. High concentrations of BG in barley have been linked with cholesterol reduction, heart well-being, and glycemic well-being, making it a healthy-directed food constituent. The fiber is also well known for its healthy digestion, healthy gastrointestinal microbiota, and immune system well-being (Rawat et al., 2023; Ahsan et al., 2024). Fatty acids play a role in barley stability as well as energy level that affect both quality in final product as well as in storage quality. The profile in barley also affects rate of lipid oxidation that can affect flavor stability in beer as well as food product shelf life (Martínez-Subirà et al., 2020; Loskutov and Khlestkina, 2021; Nowak et al., 2023). Certain FA also have established healthy attributes that play a role in cardiovascular functionality as well as metabolic balance. Excessive oxidation of lipids may also account for rancidity and off-flavor and is therefore one of the major concerns for barley improvement (Derakhshani et al., 2020; Afzal et al., 2024; Kukoeva et al., 2024). Moisture content is important both for post-harvest storage and for processing efficiency. High moisture content grains may be prone to mold growth, spoilage, and grain deterioration, whereas very dry grains may be brittle and non-processible.

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Dissecting the genetic basis of these constituents is critical in order to target breeding (Yang et al., 2019; Iannucci et al., 2021; Thabet et al., 2022).

Recombinant Inbred Lines (RILs) are a great source in exploring genetic architecture involved in complex traits. Obtained from a two-line crossing that is contrasting in target traits, RILs are a consistent genetic material that can be utilized in mapping quantitative trait loci (QTLs) involved in these traits (Hosseinian et al., 2008; Derakhshani, 2019; Huang et al., 2024). In barley quality in grains, RIL is important in determining involved genes and regions in the genome involved in accumulating key nutrient functional constituents. The information can be utilized in designing molecular markers that can be used in marker-assisted selection (MAS) in breeding (Zeng et al., 2020; Meng et al., 2023; Eid et al., 2024).

Environmental factors, i.e., climatic condition as well as soil condition, also play a vital role in modulating those genes that are involved in grain quality traits. Fluctuations in environmental conditions from location to location as well as from year to year can lead to extreme fluctuations in grain quality in a single barley genotype (Jin et al., 2022; Friero et al., 2024; Yiblet et al., 2024). Therefore, it is necessary that RIL performances be compared in a variety of contrasting environments in order to measure stability as well as heritability in targeted traits. In this way, environmental impacts can be ruled out from genetic impacts (Hambira, 2010; Niazi et al., 2022; Baloch et al., 2024).

This study aimed to analyze the differences in functional nutrient content among grains in a collection of barley RILs. To achieve this, we (1) examined the variability in functional nutrient content among the RILs, (2) measured the influence of genetic and environmental factors on these traits, and (3) explored the relationships between different quality traits in the grains. The findings provided insights into the genetic control of barley grain quality and contributed valuable information for barley breeding programs focused on improving nutritional value and end-use quality.

Materials and methods

Plant material

This study utilized a population of 193 recombinant inbred lines (RILs) derived from a cross between two barley cultivars: Ziguangmangluoerleng (ZGMLEL) and Schooner. The parental lines, ZGMLEL and Schooner, were also included in the study, bringing the total number of genotypes evaluated to 195.

Field experiment

The 195 barley genotypes (193 RILs + 2 parents) were grown in field trials during three consecutive years: 2021, 2022, and 2023. The experiment was conducted at the Songming Research station of Yunnan Academy of Agricultural Sciences in Xiaojie Town, Songming County, Yunnan Province (25°7'29" north latitude, 102°45'44" east longitude). A randomized complete block design with three replications was used. The field with good irrigation and drainage and uniform soil fertility was selected, 2 m row length, 100 grains per row, 0.30 m row spacing, and arranged in sequence. Management according to local conventional agricultural practices.

Trait measurement

Seven key nutrient functional components were evaluated in the barley Trait Measurement: Barley grains were dried in the sun, and 30 g samples of each genotype were taken. One 30 g portion was ground into a fine powder using a grinder, dried, and stored at low temperature. This powdered sample was then sieved, placed in a sealed bag, and numbered for subsequent analysis. A separate 30 g portion of unground seeds was also placed in a sealed bag and numbered. For near-infrared (NIR) analysis, a 44 cm^2 standard sample dish was used. Each sample (powdered or whole grain, as appropriate for the specific trait being measured) was placed in the dish, leveled, and compacted to create a smooth, uniform surface that did not extend beyond the dish's upper edge. The sample dishes were then sequentially analyzed using a near-infrared analyzer. The average value of three replicates was recorded for each sample. NIR calibration models for each trait (moisture, amylose, starch, BG, FA, and protein content) were internally developed using a representative subset of barley samples. These samples were analyzed through conventional wet chemistry methods to generate reference data. The calibration models were validated using independent test sets and evaluated based on the coefficient of determination ($R^2 > 0.85$), standard error of calibration (SEC), and residual predictive deviation (RPD > 2.5), indicating acceptable accuracy and predictive reliability for high-throughput phenotyping. This NIR analysis provided percentage values for moisture, amylose, starch, BG, FA, and protein content. The spectral data generated by the NIR analysis were also exported for further analysis. The content of Tac in barley grains were measured using the pH differential method (Hosseinian et al., 2008).

Statistical analysis

Analysis of variance (ANOVA) was performed to assess the effects of genotype, year, and their interaction on each of the measured traits (Steel and Torrie, 1986). Mean of superior genotypes for studied traits compared with their parent using the following formula Difference (%) = (GM-PM)/PM X100) where GM (genotypes mean) and PM (parent mean). Correlation analysis was used to examine the relationships between the studied traits.

Results and discussion

Total anthocyanin (Tac)

Anthocyanins in barley grain contribute to its antioxidant capacity, potentially enhancing its health benefits for consumers and adding value to specialized food products. Higher Tac can also influence grain color, which may be desirable for certain applications (Hosseinian et al., 2008; Yang et al., 2013). The ANOVA revealed highly significant ($p < 0.01$) effects of year and genotype on Tac content (*Table 1*). This indicates that Tac levels are influenced by both the growing year and the specific genotype. However, the year-by-genotype interaction was not significant (ns), suggesting that the relative performance of genotypes for Tac content was consistent across the three years. While the absolute Tac values might differ due to yearly environmental variations, the ranking of genotypes remained relatively stable (Hosseinian et al., 2008; Shvachko et al., 2021). In the present study, Tac content in the barley genotypes exhibited notable variability. Across the three years, the minimum Tac levels remained consistently low, ranging from 0.14 in 2022 to 0.28 in 2023, suggesting a baseline presence of these compounds. However, the maximum Tac content fluctuated considerably, from 1.09 in 2022 to 1.57 in 2023, with the highest observed in 2023 (*Table 2*). This indicates that certain genotypes or environmental conditions in that year were more conducive to anthocyanin production. The mean Tac content was also variable, with 2023 showing the highest average (0.73) and 2022 the lowest (0.46). While the absolute differences in Tac might seem small, the relatively high coefficient of variation (16.49% in 2021, 17.42% in 2022 and 18.88% in 2023) highlights the significant proportional variability, which is crucial for selection purposes. This suggests that even small increases in Tac could be meaningful (Hosseinian et al., 2008; Amer et al., 2012; Shvachko et al., 2021).

Table 1. *Mean sum of squares values form ANOVA for studied traits for 195 barely genotypes in different years*

Sources of Variations	Df	Tac	Moisture	Amylose	Starch	BG	FA	Protein
Replication	2	349.21	82.69	219.1	408.81	3.79	1.189	0.656
Years	2	162.65**	768.97**	14806.1**	533.05**	143.46**	748.01**	236.67**
Genotype	194	46.13**	171.36*	421.8**	20.28*	31.09*	438.179**	13.21*
Years*Genotypes	388	09.86^{ns}	4.86^{ns}	24.4^{ns}	11.03^{ns}	9.1^{ns}	14.098^{ns}	0.046^{ns}
Error	1168	8.922	27.93	190	9.43	4.46	1.037	0.056
Total	1754							

Mean sum of squares from analysis of variance (ANOVA) for seven functional grain traits (total anthocyanin (Tac), moisture, amylose, starch, β-glucan (BG), fatty acid (FA), and protein content) across three years (2021–2023) in 195 barley genotypes (193 RILs + 2 parents). Significance levels are denoted as *p < 0.05, **p < 0.01, ns = non-significant. Replication, genotype, year, and genotype × year interactions are included as sources of variation

Table 2. *Estimation of descriptive parameters for the functional components of 195 barley genotypes*

Parameters	Year	Tac	Moisture	Amylose	Starch	BG	FA	Protein
Minimum	2021	0.21	10.66	18.43	45.04	3.83	0.60	15.40
	2022	0.14	10.46	18.52	47.12	3.90	0.08	14.44
	2023	0.28	11.06	15.00	44.44	3.60	0.38	17.22
maximum	2021	1.35	12.49	27.02	55.80	13.05	1.95	22.43
	2022	1.09	11.89	26.42	55.66	12.54	1.85	21.52
	2023	1.57	11.83	22.75	54.46	10.86	1.84	23.81
Mean	2021	0.55	11.28	21.62	51.58	9.01	1.10	18.42
	2022	0.46	11.24	22.06	51.76	9.29	1.01	17.56
	2023	0.73	11.43	18.77	49.93	6.79	0.90	20.18
Variances	2021	0.04	0.08	1.99	4.99	2.80	0.05	2.01
	2022	0.04	0.07	1.98	4.01	2.13	0.08	1.99
	2023	0.06	0.02	1.89	3.85	2.31	0.10	1.56
Standard Deviation	2021	0.09	0.29	1.41	2.23	1.67	0.19	1.42
	2022	0.08	0.26	1.41	2.00	1.46	0.19	1.41
	2023	0.14	0.14	1.37	1.96	1.21	0.17	1.25
Coefficient of Variation	2021	16.49	2.58	6.52	4.33	18.56	17.24	7.69
	2022	17.42	2.31	6.38	3.87	15.70	18.84	8.03
	2023	18.88	1.25	7.32	3.93	17.80	18.95	6.19

Descriptive statistics (minimum, maximum, mean, variance, standard deviation, and coefficient of variation) for seven functional components of barley grain measured in 195 genotypes during 2021–2023. The traits include total anthocyanin (Tac), moisture, amylose, starch, β-glucan (BG), fatty acid (FA), and protein content

Genotype z79 consistently ranked among the top performers for Tac across all three years, exhibiting the highest Tac content: 1.35 in 2021, 1.09 in 2022, and 1.567 in 2023 (*Table 3*). This makes z79 a particularly promising candidate for breeding programs aimed at enhancing barley grain anthocyanin levels. The significant improvement over the parental mean (PM) in each year, with percentage differences reaching as high as 94.22% in 2023, suggests that this genotype possesses favorable alleles for anthocyanin biosynthesis. Other genotypes, such as z19, z15, z34, and z95, also frequently appeared in the top 10, though their rankings fluctuated. The overall genotypic mean (GM) for Tac was consistently higher than the parental means across all years, indicating the effectiveness of selection for this trait. The higher Tac values observed in 2023 may be attributed to environmental factors like increased solar radiation or temperature fluctuations, which are known to influence anthocyanin production in plants (Han et al., 2018; Xiong et al., 2022). Anthocyanins are potent antioxidants that contribute to the health benefits of barley. The negative correlation between starch and anthocyanin across all years indicates a metabolic trade-off between energy storage and antioxidant biosynthesis. This finding is crucial for breeders aiming to balance yield and functional quality, as selecting for high starch may inadvertently reduce health-promoting traits like anthocyanins (Jin et al., 2022; Afzal et al., 2024).

Moisture

Appropriate moisture levels in barley grain are essential for maintaining its quality during storage, preventing mold growth, and ensuring optimal milling and malting performance. Accurate moisture content is critical for trade and processing (Mohamed et al., 2010; Graton et al., 2024; Huang et al., 2024). In this study, Year had a highly significant ($p < 0.01$) effect on moisture content, demonstrating the strong influence of environmental conditions on this trait (*Table 1*). Genotype also had a significant ($p < 0.05$) effect, indicating genetic variation for moisture content among the barley genotypes. The non-significant (ns) year-by-genotype interaction suggests that the genotypes maintained relatively consistent moisture levels relative to each other across the three years, similar to what was observed for Tac. Moisture content proved to be a remarkably stable trait across the three years. Both minimum and maximum moisture levels varied only slightly, ranging from 10.46% to 11.06% for the minimum and 11.83% to 12.49% for the maximum. The mean moisture content was also quite consistent, with 11.28% in 2021, 11.24% in 2022, and 11.43% in 2023 (*Table 2*). The extremely low variance (0.08 in 2021, 0.07 in 2022, and 0.02 in 2023), standard deviation (0.29 in 2021, 0.26 in 2022, and 0.14 in 2023), and coefficient of variation (2.58% in 2021, 2.31% in 2022, and 1.25% in 2023) confirm this stability, indicating that moisture content is likely less influenced by either genotype or environmental fluctuations compared to the other traits (Ahokas et al., 2018; Jin et al., 2022; Niazi et al., 2022).

The top-performing genotypes for moisture content varied more across the three years compared to Tac. While z126 showed high moisture levels in both 2021 (12.49) and 2022 (11.89), different genotypes dominated the top 10 list in 2023, with z103 exhibiting the highest moisture content (11.83) (*Table 3*). The genotypic means for moisture were consistently higher than the parental means, though the percentage differences were relatively small, ranging from 3.94% to 8.24%. Genotypes z146 and z63 appeared in the top 10 for two of the three years.

Table 3. *Top 10 best performing genotypes in studied year 2021, 2022, 2023 and comparison with their parents*

Traits	Year	10 Best performing genotypes out of 195 genotypes selected based on the mean values and performance	GM	PM	Differences (%)
Tac	2021	z79 (1.35), followed by z27 (1.23), z19 (1.18), z159 (1.1), z15 (1.08), z34 (1.08), z35 (1.06), z95 (1.02), z138 (1) and z102 (0.98),	1.11	0.68	62.37
	2022	z79 (1.09), followed by z19 (1.03), z34 (0.98), z95 (0.95), z15 (0.94), z60 (0.93), z97 (0.86), z52 (0.85), z37 (0.82), and z45 (0.82),	0.93	0.53	74.45
	2023	z79 (1.567), followed by z138 (1.507), z15 (1.394), z19 (1.373), z52 (1.357), z167 (1.347), z147 (1.288), z17 (1.264), z95 (1.243), z159 and (1.205),	1.35	0.70	94.22
Moisture	2021	z126 (12.49), followed by z43 (12.42), z127 (12.39), z7 (12.26), z146 (12.26), z12 (12.22), z9 (12.08), z143 (12.03), z13 (12.01), z149 and (11.94),	12.21	11.28	8.24
	2022	z126 (11.89), followed by z63 (11.86), z95 (11.82), z96 (11.81), z138 (11.81), z140 (11.77), z4 (11.7), z189 (11.69), z91 (11.68), z132 and (11.66),	11.77	11.08	6.23
	2023	z103 (11.83), followed by z146 (11.79), z85 (11.78), z63 (11.77), z153 (11.75), z152 (11.73), z3 (11.71), z114 (11.7), z147 (11.7), z124 and (11.69),	11.75	11.31	3.94
Amylose	2021	z7 (27.02), followed by z9 (25.89), z14 (24.95), z12 (24.82), z17 (24.59), z13 (24.33), z89 (24.26), z11 (24.24), z56 (24.06), z1 (24.03),	24.82	20.47	21.25
	2022	z11 (26.42), followed by z4 (25.63), z9 (25.6), z156 (25.39), z19 (25.33), z162 (25.12), z74 (25), z154 (24.98), z17 (24.87), z15 (24.67),	25.30	22.01	14.95
	2023	z154 (22.75), followed by z162 (22.56), z156 (22.35), z170 (22.35), z171 (21.48), z70 (21.36), z174 (21.15), z90 (21.14), z189 (21.06), z11 and (21.04),	21.72	19.13	13.57
starch	2021	z84 (55.8), followed by z71 (55.1), z148 (55.06), z55 (55.03), z86 (55.01), z90 (54.92), z140 (54.67), z170 (54.66), z184 (54.61), z66 and (54.58),	54.94	49.87	10.17
	2022	z1 (50.39), followed by z2 (52.6), z3 (52.22), z4 (49.04), z5 (50.56), z6 (51.64), z7 (48.04), z8 (50.67), z9 (51.67), and z10 (51.81),	50.86	50.87	-0.01
	2023	z146 (54.46), followed by z90 (54.18), z148 (54.15), z63 (54), z84 (53.54), z140 (53.53), z47 (53.51), z114 (53.04), z2 (52.84), z189 and (52.75),	53.60	47.86	12.01
BG	2021	z9 (13.05), followed by z11 (12.59), z12 (11.9), z26 (11.77), z7 (11.53), z36 (11.52), z154 (11.46), z4 (11.12), z27 (11.12), z146 and (11.12),	11.72	7.70	52.31
	2022	z192 (12.54), followed by z4 (12.39), z190 (12.25), z11 (12.16), z189 (12.02), z19 (11.7), z6 (11.64), z193 (11.62), z122 (11.53), z117 and (11.47),	11.93	10.06	18.59
	2023	z189 (10.86), followed by z187 (10.67), z90 (10.25), z88 (10.23), z188 (9.83), z84 (9.82), z86 (9.69), z92 (9.61), z190 (9.58), and z193 (9.56),	10.01	8.34	20.10
FA	2021	z95 (1.95), followed by z85 (1.62), z13 (1.6), z96 (1.58), z138 (1.58), z43 (1.56), z105 (1.56), z127 (1.5), z133 (1.5), and z37 (1.48),	1.59	1.15	38.26

Traits	Year	10 Best performing genotypes out of 195 genotypes selected based on the mean values and performance	GM	PM	Differences (%)
	2022	z61 (1.85), followed by z63 (1.82), z62 (1.8), z155 (1.79), z153 (1.75), z79 (1.6), z138 (1.58), z159 (1.54), z161 (1.53), and z157 (1.52),	1.68	0.76	121.05
	2023	z167 (1.84), followed by z85 (1.82), z153 (1.76), z172 (1.67), z155 (1.65), z150 (1.64), z154 (1.59), z161 (1.58), z175 (1.57), and z151 (1.56),	1.67	1.22	36.89
Protein	2021	z104 (22.43), followed by z159 (22.11), z27 (21.97), z79 (21.91), z35 (21.72), z34 (21.71), ZGMLEL(P1) (21.61), z117 (21.53), z99 (20.84), and z102 (20.84),	21.67	19.60	10.56
	2022	z174 (21.52), followed by z175 (21.47), z172 (21.23), ZGMLEL(P1) (20.62), z34 (20.6), z99 (20.52), z59 (20.19), z173 (19.99), z56 (19.91), and z60 (19.89),	20.59	17.95	14.71
	2023	z108 (23.81), followed by z79 (23.77), z99 (23.43), z3 (22.91), z104 (22.89), z120 (22.65), z121 (22.65), ZGMLEL(P1) (22.65), z59 (22.62), and z56 (22.56),	22.99	20.81	10.50

Top 10 barley genotypes selected based on highest performance for each trait (Tac, moisture, amylose, starch, β-glucan, fatty acid, and protein) across the years 2021, 2022, and 2023. GM: Mean value of the top 10 genotypes; PM: Mean value of the parent genotypes; Difference (%): Percent increase of GM over PM

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Grain moisture content is crucial for storability and processing. While the observed variations are relatively small, they can still impact grain quality. The influence of environmental factors on moisture content is well-documented, as water availability and temperature during grain filling can significantly affect final moisture levels (Hambira, 2010; Martínez-Subirà et al., 2020; Raj et al., 2023).

Amylose

Amylose content in barley grain affects its cooking quality and digestibility, influencing its suitability for different food products like noodles, bread, or as a source of resistant starch. Specific amylose levels are often targeted for particular end-uses. The ANOVA showed highly significant ($p < 0.01$) effects of year and genotype on amylose content (*Table 1*). This indicates that amylose content is influenced by both genetic and environmental factors. However, the non-significant (ns) year-by-genotype interaction suggests that the relative performance of genotypes for amylose content was consistent across years. Amylose content showed moderate variability across the three years. The minimum amylose content ranged from 15.00 in 2023 to 18.52 in 2022, while the maximum ranged from 22.75 in 2023 to 27.02 in 2021 (*Table 2*). The mean amylose content was highest in 2022 (22.06) and lowest in 2023 (18.77). The moderate variance (1.99 in 2021, 1.98 in 2022, and 1.89 in 2023), standard deviation (1.41 in both 2021 and 2022, and 1.37 in 2023), and coefficient of variation (6.52% in 2021, 6.38% in 2022, and 7.32% in 2023) suggest that amylose content is subject to some degree of genotypic and/or environmental influence, though less so than Tac or BG (Xue et al., 2016; Derakhshani, 2019; Huang et al., 2024).

Genotype z7 exhibited the highest amylose content in 2021 (27.02), while z11 topped the list in 2022 (26.42). In 2023, z154 had the highest amylose content at 22.75. Several genotypes, like z9, z17, and z11, consistently appeared in the top 10 for at least two of the three years (*Table 3*). The genotypic means for amylose were substantially higher than the parental means across all years, with percentage differences ranging from 13.57% to 21.25%. Amylose content is a key determinant of barley's end-use quality, affecting cooking properties and digestibility. The observed year-to-year variation could be related to temperature during grain development, as higher temperatures are often associated with lower amylose content. The higher amylose content in the RILs compared to the parents suggests the presence of favorable alleles for amylose synthesis in the RIL population (Hambira, 2010; Loskutov and Khlestkina, 2021; Jin et al., 2022).

Starch

Starch is the primary component of barley grain, providing the major source of energy. The quantity and type of starch impact its nutritional value and its use in brewing, food processing, and animal feed. Year and genotype had significant ($p < 0.05$) effects on starch content, indicating the importance of both genetic and environmental factors in determining starch levels in barley grain (*Table 1*). The non-significant (ns) year-by-genotype interaction suggests that the relative ranking of genotypes for starch remained consistent across the three years. Starch content demonstrated relative stability across the three years. The minimum starch content ranged from 44.44% in 2023 to 47.12% in 2022, while the maximum ranged from 54.46% in 2023 to 55.80% in 2021 (*Table 2*). The mean starch content also showed minimal variation, with 51.58% in 2021, 51.76% in 2022, and 49.93% in 2023. The low variance (4.99 in 2021, 4.01 in 2022, and 3.85 in 2023), standard deviation (2.23 in 2021, 2.00 in 2022, and 1.96 in 2023), and coefficient of variation

(4.33% in 2021, 3.87% in 2022, and 3.93% in 2023) further support this stability, implying that starch content is a relatively consistent trait in these barley genotypes (Derakhshani, 2019; Raj et al., 2023; Yiblet et al., 2024).

The top-performing genotypes for starch content varied considerably across the three years. z84 had the highest starch content in 2021 (55.8), while z1 led the list in 2022 (50.39), and z146 topped the list in 2023 (54.46) (*Table 3*). Few genotypes consistently ranked high across all three years. The genotypic means for starch showed improvement over the parental means in 2021 and 2023, with percentage differences of 10.17% and 12.01%, respectively. However, in 2022, the genotypic and parental means were nearly identical. Genotypes z90 and z148 appeared in the top 10 in two of the three years. Starch is the primary source of energy in barley grain. The observed fluctuations in starch content across years could be due to variations in temperature and water availability during grain fill. The lack of consistent high performers across years emphasizes the strong environmental influence on starch accumulation (Yang et al., 2013; Xue et al., 2016).

β-glucan (BG)

β-glucans in barley grain are valuable dietary fibers with demonstrated health benefits, such as lowering cholesterol. Higher BG content in barley grain enhances its nutritional profile and potential for use in functional foods. The ANOVA revealed highly significant ($p < 0.01$) effects of year and genotype on BG content (*Table 1*). This demonstrates that BG content is subject to both genetic and environmental control. The non-significant (ns) year-by-genotype interaction indicates that the relative BG content of the genotypes was consistent across the three years. BG content displayed substantial variability across the three years. The minimum BG content ranged from 3.60 in 2023 to 3.90 in 2022, while the maximum ranged from 10.86 in 2023 to 13.05 in 2021 (*Table 2*). The mean BG content also varied, with 9.29 in 2022, 9.01 in 2021, and 6.79 in 2023. The high variance (2.80 in 2021, 2.13 in 2022, and 2.31 in 2023), standard deviation (1.67 in 2021, 1.46 in 2022, and 1.21 in 2023), and coefficient of variation (18.56% in 2021, 15.70% in 2022, and 17.80% in 2023) underscore the significant variability in BG content, indicating a strong influence of genotype and/or environmental factors on the accumulation of this compound (Hambira, 2010; Zeng et al., 2020; Niazi et al., 2022).

Genotype z9 exhibited the highest BG content in 2021 (13.05), while z192 led in 2022 (12.54), and z189 had the highest BG content in 2023 (10.86) (*Table 3*). While some genotypes appeared in the top 10 for multiple years, the overall ranking and the range of BG values within the top 10 varied considerably. The genotypic means for BG were substantially higher than the parental means across all years, with percentage differences ranging from 18.59% to 52.31%. Genotypes z11, z4, and z189 were present in the top 10 for at least two of the three years. BG are valuable dietary fibers. The significant variation observed in BG content highlights the complex interaction between genotype and environment. Environmental factors like temperature and water stress during grain development can influence BG synthesis (Derakhshani et al., 2020; Martínez-Subirà et al., 2020; Eid et al., 2024).

Fatty acid (FA)

Fatty acid content in barley grain contributes to its overall nutritional composition and can influence its stability during storage. FA profiles are important considerations for feed and food applications. Year had a highly significant ($p < 0.01$) effect, and genotype had a highly significant effect on FA content, indicating that FA content is influenced by both genetic and environmental factors (*Table 1*). The non-significant (ns) year-by-genotype interaction suggests that the relative ranking of genotypes for FA content remained consistent across the three years. FA content exhibited considerable relative variation, despite the low absolute values. The minimum FA content showed a dramatic drop in 2022 (0.08) compared to 2021 (0.60) and 2023 (0.38). While the maximum FA content remained relatively consistent, ranging from 1.84 in 2023 to 1.95 in 2021, the mean FA content varied, with 1.10 in 2021, 1.01 in 2022, and 0.90 in 2023 (*Table 2*). The high coefficient of variation (17.24% in 2021, 18.84% in 2022, and 18.95% in 2023), coupled with the low means, emphasizes the importance of considering this trait carefully, as even small changes in FA content could be proportionally significant (Derakhshani et al., 2020; Friero et al., 2024; Yiblet et al., 2024).

Genotype z95 had the highest FA content in 2021 (1.95), while z61 topped the list in 2022 (1.85), and z167 led in 2023 (1.84). Similar to the other traits, the top-performing genotypes for FA varied across the years. The genotypic means for FA were consistently higher than the parental means, with percentage differences ranging from 36.89% to 121.05% (*Table 3*). This indicates substantial improvement in FA content in the RIL population compared to the parents. This contribute to the nutritional profile and storage stability of barley grain. The large percentage differences between the RILs and the parents suggest that the RIL population is enriched with genotypes carrying alleles that promote higher FA accumulation (Martínez-Subirà et al., 2020; Ahsan et al., 2024).

Protein

Protein content in barley grain is a crucial factor for its nutritional value, especially when used for food or animal feed. Protein levels influence malting quality for brewing and are important in determining market value. In this study, year and genotype both had significant ($p < 0.05$) effects on protein content, signifying the complex interplay of genetic and environmental factors in determining protein levels (*Table 1*). The non-significant (ns) year-by-genotype interaction suggests that the relative protein content of the genotypes remained consistent across the three years. Protein content showed moderate variability across the three years. The minimum protein content ranged from 14.44 in 2022 to 17.22 in 2023, while the maximum ranged from 21.52 in 2022 to 23.81 in 2023 (*Table 2*). The mean protein content was highest in 2023 (20.18) and lowest in 2022 (17.56). The moderate variance (2.01 in 2021, 1.99 in 2022, and 1.56 in 2023), standard deviation (1.42 in 2021, 1.41 in 2022, and 1.25 in 2023), and coefficient of variation (7.69% in 2021, 8.03% in 2022, and 6.19% in 2023) suggest that protein content is influenced by both genotype and environment, though to a lesser extent than Tac or BG (Hambira, 2010; Martínez-Subirà et al., 2020; Nowak et al., 2023).

Genotype z104 had the highest protein content in 2021 (22.43), while z174 led in 2022 (21.52), and z108 topped the list in 2023 (23.81). The parental line ZGMLEL(P1) appeared in the top 10 for all three years, though its ranking varied (*Table 3*). The genotypic means for protein were consistently higher than the parental means across all years, with percentage differences ranging from 10.50% to 14.71%. Genotypes z79 and z99 also appeared in the top 10 for all three years. Protein content is a crucial nutritional parameter. The relatively consistent performance of z79 and z99 genotypes across years suggests a degree of genetic control over protein accumulation. Nitrogen availability in the soil is a key environmental factor known to influence protein content in grains (Yang et al., 2019; Victoria et al., 2023; Eid et al., 2024).

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The current results of ANOVA indicated that while the absolute levels of each trait might change from year to year (due to the significant year effect), the relative ranking of the genotypes for each trait remained consistent across the three years. This is important information for breeders, as it suggests that selection for these traits can be effective, even if the absolute values are influenced by the environment. Based on their consistent performance and significant improvement over the parental means across multiple traits and years, several genotypes emerge as promising candidates for future breeding programs. Genotypes z79 (high Tac, protein), z11 (high amylose, BG), z95 (high Tac, FA), z146 (high moisture, starch), and ZGMLEL(P1) (consistent protein) demonstrate desirable characteristics for multiple traits. These genotypes, particularly z79, combine high levels of valuable components like anthocyanins and protein, making them especially attractive for developing nutritionally enhanced barley varieties. Further investigation of these genotypes, including genetic analysis, would be beneficial to fully understand their potential for barley improvement (Adriana et al., 2015; Xue et al., 2016; Yiblet et al., 2024).

Correlation analysis

The correlation analysis of the studied traits over three years (2021, 2022, and 2023) reveals dynamic relationships among biochemical components in the samples *Table 4* and *Figure 1*. The variations in correlation values across years suggest environmental or genetic influences on trait associations. The *Figure 1 (A, B, and C)* presented a correlation matrix in the form of a pie chart correlation plot, which visualized the relationships between several key biochemical traits in barley. The color gradient scale at the bottom of *Figure 1* provided a visual representation of the Pearson correlation coefficients between different pairs of traits. Red shades signified negative correlations, indicating that as one trait increased, the other tended to decrease. In contrast, blue shades indicated positive correlations, where an increase in one trait was accompanied by an increase in the other. The intensity of the color correlated with the strength of the correlation: darker shades denoted stronger correlations, while lighter shades indicated weaker relationships. The scale spanned from -1 (indicating a perfect negative correlation) to +1 (indicating a perfect positive correlation), with 0 representing no correlation at all.

Each pie chart within the matrix represented the Pearson correlation coefficient between two traits, offering a visual summary of the relationship between them. The filled portion of each pie chart reflected the strength of the correlation: a larger filled area corresponded to a stronger correlation. The color of the filled area indicated the direction of the correlation, with red representing negative correlations and blue representing positive correlations. The pie charts were organized in a symmetrical matrix, with each pair of traits compared only once. The lower triangular portion of the matrix was shown for all the studied traits.

Total anthocyanin (Tac) exhibited a negative correlation with moisture and amylose, but the significance of these associations weakened over time. A strong negative correlation was consistently observed between Tac and starch (r = -0.74** in 2021 and 2022; -0.51** in 2023), suggesting that increased starch content may suppress anthocyanin biosynthesis (*Table 4*). Tac showed no significant relationship with β-glucan (BG) or fatty acids (FA) across years. However, it maintained a strong positive correlation with protein (r = 0.69** to 0.43**), indicating a potential link between protein accumulation and anthocyanin synthesis (Hambira, 2010; Derakhshani et al., 2020; Eid et al., 2024). Moisture content correlated positively with amylose, starch, BG, and FA

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during 2021 and 2022, but these relationships weakened or became non-significant in 2023. In contrast, moisture showed a consistent negative correlation with protein across all years (r ranging from -0.30* to -0.13ns), suggesting that higher moisture may reduce protein content (Han et al., 2018; Oluwajuyitan et al., 2021; Eid et al., 2024).

Table 4. *Correlation among studied traits for the experiment conducted during the years 2021, 2022 and 2023*

Traits	Year	Total Anthocyanin	Moisture	Amylose	Starch	β-glucan	*Fatty acid*
Moisture	2021	-0.25*					
	2022	-0.03ns					
	2023	-0.01ns					
Amylose	2021	-0.18*	0.22*				
	2022	-0.17*	0.20*				
	2023	-0.12ns	-0.12ns				
Starch	2021	-0.74**	0.22*	0.13ns			
	2022	-0.74**	0.17*	0.24*			
	2023	-0.51**	0.25*	0.35**			
β-glucan	2021	-0.08ns	0.17*	0.34*	0.17*		
	2022	0.03ns	0.21*	0.30*	0.09ns		
	2023	-0.08ns	0.04ns	0.34**	0.27*		
Fatty acid	2021	0.13*	0.49**	-0.27*	-0.04ns	-0.37*	
	2022	-0.02ns	0.42**	-0.06ns	0.12	-0.55**	
	2023	0.08ns	0.08ns	0.24*	-0.18*	0.08ns	
Protein	2021	0.69**	-0.30*	-0.39**	-0.90**	-0.25*	0.02**
	2022	0.64**	-0.24*	-0.53*	-0.85*	-0.05ns	-0.20*
	2023	0.43**	-0.13ns	-0.53**	-0.82**	-0.25**	-0.07ns

Pearson correlation coefficients among functional grain traits in 195 barley genotypes measured over three years (2021–2023). Traits include total anthocyanin (Tac), moisture, amylose, starch, β-glucan (BG), fatty acid (FA), and protein. Significance levels: *p < 0.05, **p < 0.01, ns = non-significant

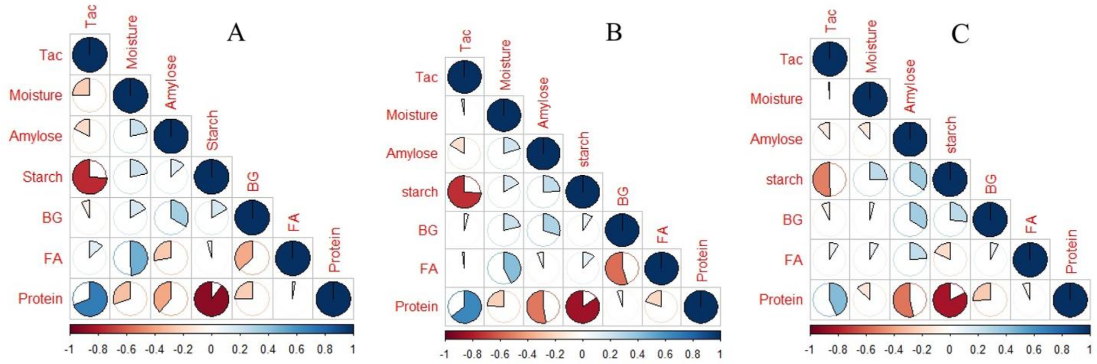

Figure 1. *Correlation Matrix of Key Biochemical Traits in Barley. This figure illustrates the relationships between seven functional traits, as assessed in the 2021, 2022 and 2023 experiment mentioned in Figure A, B and C respectively. The matrix is composed of pie charts representing the Pearson correlation coefficients between each pair of traits, with color intensity indicating the strength and direction of correlations. Red shades indicate negative correlations, while blue shades indicate positive correlations. Larger filled portions of the pie charts signify stronger correlations. The lower triangular portion of the matrix is shown to avoid redundancy*

Amylose was positively correlated with starch and BG throughout the study, with the strongest correlation (r = 0.35**) recorded in 2023. Its relationship with FA shifted from negative in 2021 (r = -0.27*) to positive in 2023 (r = 0.24*), while its association with protein remained strongly negative across years (r = -0.39** to -0.53**) (Mohamed et al., 2010; Afzal et al., 2013; Xiong et al., 2022; Graton et al., 2024). Starch showed strong negative correlations with Tac and protein throughout, while its positive association with amylose strengthened over time. It exhibited weak and inconsistent correlations with BG and FA, indicating environmental or genotypic modulation of these traits (Zeng et al., 2020; Niazi et al., 2022; Yiblet et al., 2024).

BG demonstrated a consistent positive correlation with amylose and a variable relationship with starch. It was negatively associated with FA in 2021 and 2022 but not in 2023, and its correlation with protein was weak and only significant in 2023 (Martínez-Subirà et al., 2020; Loskutov and Khlestkina, 2021; Rawat et al., 2023). FA content was positively correlated with moisture and showed a dynamic relationship with amylose and starch—changing from negative to positive correlations over the three years. Its association with BG was negative in the first two years but disappeared in 2023. Correlations with protein were weak and inconsistent across all years (Han et al., 2018; Yang et al., 2019; Geng et al., 2022). Protein content was strongly and positively associated with Tac, while maintaining significant negative correlations with starch, amylose, and moisture. Its relationships with BG and FA were weaker and more variable, suggesting these traits are less tightly linked with protein accumulation (Irshad et al., 2024; Teoh et al., 2024).

One of the key findings from the correlation analysis is the consistently strong negative relationship between starch and protein content across all study years. This inverse association suggests that an increase in starch accumulation comes at the expense of protein synthesis, indicating a competitive allocation of carbon and nitrogen resources during grain development (Thabet et al., 2022; Kukoeva et al., 2024). From a breeding perspective, this trade-off highlights the challenge of simultaneously optimizing yield-related traits (like starch) and nutritional quality (such as protein content). A similar and persistent negative correlation was observed between starch and total anthocyanin (Tac), reinforcing the notion that genotypes favoring starch deposition may suppress anthocyanin biosynthesis—compounds associated with antioxidant and defense functions (Hosseinian et al., 2008; Iannucci et al., 2021). Selecting for high-starch lines, therefore, must be carefully balanced to avoid compromising antioxidant potential and grain pigmentation.

Moisture content showed a weakening negative correlation with Tac over time, with the strongest relationship detected in 2021. This temporal pattern suggests that anthocyanin accumulation may have been more sensitive to specific environmental factors—such as humidity and temperature—during that year. Additionally, moisture was positively associated with starch and amylose in the earlier years but less so in 2023, implying that the influence of water availability on carbohydrate synthesis is modulated by seasonal variation and genotypic response (Hosseinian et al., 2008; Eid et al., 2024). The consistently positive correlation between amylose and β-glucan (BG) across all years implies shared biosynthetic regulation, likely due to their mutual role in dietary fiber content and functional food properties. This is promising for breeders aiming to enhance health-promoting components, as selecting for higher amylose content may simultaneously increase BG levels—a valuable trait in improving cereal-based functional foods (Derakhshani, 2019; Thabet et al., 2022). On the other hand, amylose exhibited a negative correlation with protein, indicating a resource allocation trade-off that limits the simultaneous enhancement of both traits. Fatty acid (FA) correlations showed year-to-year fluctuations, particularly in relation to moisture, amylose, and BG. In 2021, FA was positively associated with moisture but negatively correlated with amylose and BG. These relationships shifted in 2022 and 2023, reflecting the influence of both genetic expression and environmental variability on lipid metabolism (Derakhshani, 2019; Zeng et al., 2020). Such dynamics underscore the importance of evaluating biochemical traits over multiple seasons to identify stable, heritable associations.

A consistently strong positive correlation between protein and Tac suggests that both may be co-regulated by nitrogen availability or share metabolic pathways, offering an opportunity to simultaneously improve nutritional value and antioxidant capacity through selection (Jin et al., 2022). Conversely, the negative correlations of protein with starch, moisture, and amylose reinforce the challenge of combining high yield with high nutritional content. These trade-offs imply that selecting for high-starch genotypes may unintentionally reduce levels of protein and anthocyanins, potentially affecting the grain's dietary quality (Ijaz et al., 2023; Kadege et al., 2024). The positive relationship between amylose and BG highlights a synergistic target for improving dietary fiber content, while the fluctuating associations between FA and other biochemical traits indicate the necessity of genotype-by-environment interaction studies. Future research should explore how variables like rainfall, temperature, and soil nutrients influence these correlations to support more precise selection in breeding programs (Fatemi et al., 2023; Hejazi et al., 2025). Overall, the findings underline the importance of a balanced breeding strategy that enhances not only yield-related traits but also quality parameters such as BG and protein, optimizing the nutritional and functional value of barley grains (Yang et al., 2013; Ahsan et al., 2024; Eid et al., 2024). These insights are valuable for guiding breeding decisions that align with both health-oriented food science and agricultural productivity goals.

Conclusion

This study provides key insights into the differences in nutrient functional composition among various grain types in barley recombinant inbred lines (RILs). Significant genetic and environmental effects were observed on Tac, moisture, amylose, starch, BG, FA, and protein content, with genotype rankings remaining stable across years, making them suitable for selection in breeding programs. Genotype z79 consistently exhibited high Tac and protein levels, with a peak Tac content of 1.57 in 2023, a 94.22% improvement over the parental mean. Moisture content remained stable (CV < 3%), while amylose (15.00%-27.02%) and starch (~51%) showed moderate fluctuations. BG content varied widely (3.60%–13.05%), indicating strong environmental influence. FA content showed proportional variation (CV: 17.24%–18.95%), and protein reached its highest level (23.81%) in 2023. Correlation analysis highlighted key trade-offs, with starch and protein showing a strong negative correlation (-0.90** to -0.82**), while Tac was inversely correlated with starch (-0.74** to -0.51**). Conversely, amylose and BG exhibited a stable positive correlation (0.34** in 2023), making them promising targets for functional food applications. Superior genotypes, including z79 (high Tac, protein), z11 (high amylose, BG), and z95 (high Tac, FA), are ideal candidates for breeding programs. These superior RILs could be strategically utilized in future breeding strategies to enhance nutritional traits, with a focus on creating barley varieties that not only offer superior nutrient profiles but also adapt well to varying environmental conditions, ultimately contributing to climate-resilient and nutritionally enhanced crops. Future studies should explore environmental factors influencing these traits to further refine breeding approaches.

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REFERENCES

[1] Adriana, C., Madosa, E., Giancarla, V., Ciulca, S. (2015): Combining ability for some grains morphological traits in winter barley. – Journal of Horticulture, Forestry and Biotechnology 19: 67-72.

[2] Afzal, G., Ullah, M. I., Ali, N., Afzal, M., Hussain, R., Alhakamy, N. A., Rajeh, N., Rehan, S., Iqbal, R., Iqbal, M. S. (2024): Mechanistic approach to investigate the induction of toxicity by magnesium oxide nanoparticles on testicular, nervous and muscular tissues of albino rats. – Asian Journal of Agriculture and Biology 4: 2024152.

[3] Ahokas, H. (2018): Barley CMS detected in Finland in 1976 enabled growing of productive winter-barley F1 hybrids in the European winter-barley zone since 2002. – Suomen Maataloustieteellisen Seuran Tiedote 1: 1-7.

[4] Ahsan, H., Ayub, M., Gul, M., Qureshi, A., Asfour, H., Bilal, H., Azeem, M., Wahid, A., Ali, N., Naveed, R. (2024): Efficacy of silver oxide nanoparticles against multi-drug resistant Pseudomonas aeruginosa and methicillin-resistant *Staphylococcus aureus* in burn wound infections. – Asian Journal of Agriculture and Biology 4: 2024096.

[5] Amer, K. A., Eid, A., Elakhdar, A., El-Shawy, E. (2012): Combining ability and heterosis in five barley genotypes for some economic traits. – Egyptian Journal of Agricultural Research 90: 105-116.

[6] Baloch, H., Sabir, I., Leghari, S., Saddiq, M., Alam, P., Khan, S., Fatima, E., Sajid, M., Raza, M., Arif, M. (2024): Moringa leaf extract enhances the growth and yield characteristics of buckwheat genotypes by modulating the biochemical and physiological activities. – Asian Journal of Agriculture and Biology 4: 2023328.

[7] Derakhshani, Z. (2019): Identification of suitable genetic resources of barley with potential for stress tolerance as functional foods. – PhD Thesis, Swinburne University of Technology.

[8] Derakhshani, Z., Malherbe, F., Panozzo, J. F., Bhave, M. (2020): Evaluation of diverse barley Cultivars and landraces for contents of four Multifunctional biomolecules with nutraceutical potential. – Current Research in Nutrition and Food Science Journal 8: 380-390.

[9] Eid, O., Salem, M. A., Mohamed, O. G., Ezzat, S., Tripathi, A., Ewida, M. A., El Sayed, A., Abdel-Sattar, E., Elkady, W. M. (2024): Metabolomic profiling of barley extracts obtained via different solvents and evaluation of their anti-inflammatory efficacy. – Food Bioscience 61: 104682.

[10] Fatemi, R., Yarnia, M., Mohammadi, S., Vand, E. K., Mirashkari, B. (2023): Screening barley genotypes in terms of some quantitative and qualitative characteristics under normal and water deficit stress conditions. – Asian Journal of Agriculture and Biology 2: 2022071.

[11] Friero, I., Macià, A., Romero, M. P., Romagosa, I., Martínez-Subirà, M., Moralejo, M. (2024): Phenolic Profile Dynamics during Grain Development in Hull-Less Food Barley Genotypes with Varying Grain Colors. – Foods 13: 1841.

[12] Geng, L., Li, M., Zhang, G., Ye, L. (2022): Barley: a potential cereal for producing healthy and functional foods. – Food Quality and Safety 6: fyac012.

[13] Graton, J., Goupille, A., Ferré, T., Offmann, B., André-Miral, C., Le Questel, J.-Y. (2024): Antioxidant properties of catechin and its 3′ O-α-glucoside: Insights from computational chemistry calculations. – Computational and Theoretical Chemistry 1236: 114608.

[14] Hambira, C. (2010): Proanthocyanidins, anthocyanins and phenolic acids in food barleys of diverse origin. – MSc Thesis, University of Manitoba.

[15] Han, Z., Zhang, J., Cai, S., Chen, X., Quan, X., Zhang, G. (2018): Association mapping for total polyphenol content, total flavonoid content and antioxidant activity in barley. – BMC Genomics 19: 1-10.

[16] Hejazi, M. A., Panahi, B. (2025): Evaluation of physio-morphological characteristics associated with drought tolerance in drought tolerance and sensitive barley cultivars. – Asian Journal of Agriculture and Biology 4: 2024233.

[17] Hosseinian, F. S., Li, W., Beta, T. (2008): Measurement of anthocyanins and other phytochemicals in purple wheat. – Food Chemistry 109: 916-924.

[18] Huang, H. J., Li, Y., Gao, X., Tian, P. J., Yangzong, N., Zhaxi, L., Chang, Z., Yin, Z., Liao, W. (2024): Metabolic profiling of highland barley (*Hordeum vulgare* var. nudum) genotypes. – Discover Applied Sciences 6: 83.

[19] Iannucci, A., Suriano, S., Codianni, P. (2021): Genetic diversity for agronomic traits and phytochemical compounds in coloured naked barley lines. – Plants 10: 1575.

[20] Ijaz, M., Afzal, A., Shabbir, G., Iqbal, J., Rafique, M. (2023): Breeding wheat for leaf rust resistance: past, present and future. – Asian Journal of Agriculture and Biology 2023(1): 2021426.

[21] Irshad, M., Ondrasek, G., Iqbal, A. (2024): Influence of anaerobic digestate type on nitrogen dynamics and leaching losses across two soils. – Asian Journal of Agriculture and Biology 4: 2024041.

[22] Jin, H.-M., Dang, B., Zhang, W.-G., Zheng, W.-C., Yang, X.-J. (2022): Polyphenol and anthocyanin composition and activity of highland barley with different colors. – Molecules 27: 3411.

[23] Kadege, E. L., Venkataramana, P. B., Assefa, T., Ndunguru, J. C., Rubyogo, J. C., Mbega, E. R. (2024): Assessing disease resistance and yield components in advanced breeding lines of common bean in different locations of northern Tanzania. – Asian Journal of Agriculture and Biology 4: 2023233.

[24] Kukoeva, T. V., Molobekova, C. A., Totsky, I. V., Vasiliev, G. V., Pronozin, A. Y., Afonnikov, D. A., Khlestkina, E. K., Shoeva, O. Y. (2024): Enrichment of Grain Anthocyanin Content through Marker-Assisted Breeding for Ant1, Ant2 or HvMyc2 Genes in Barley (*Hordeum vulgare* L.). – Agronomy 14: 1231.

[25] Liaqat, S., Cai, K., Saddique, M. A. B., Sher, M. A., Ali, Z. (2024): Barley biofortification for food security: challenges and future prospects. – In: Biofortification of Grain and Vegetable Crops, Chapter 6, pp. 97-114.

[26] Loskutov, I. G., Khlestkina, E. K. (2021): Wheat, barley, and oat breeding for health benefit components in grain. – Plants 10: 86.

[27] Martínez-Subirà, M., Romero, M. P., Puig, E., Macià, A., Romagosa, I., Moralejo, M. (2020): Purple, high β-glucan, hulless barley as valuable ingredient for functional food. – Lwt 131: 109582.

[28] Meng, G., Rasmussen, S. K., Christensen, C. S., Fan, W., Torp, A. M. (2023): Molecular breeding of barley for quality traits and resilience to climate change. – Frontiers in Genetics 13: 1039996.

[29] Mohamed, A. A., Matter, M. A., Rady, M. R. (2010): Assessment of some barley germplasms based on RAPD analysis and anti-nutritional factors. – Journal of Crop Science and Biotechnology 13: 61-68.

[30] Niazi, M. K., Hassan, F., Imran, S., Saeed, Z., Riaz, K., Sohail, Z., Ismail, M. A. (2022): Potential Therapeutic Effect of Barley on Cardiovascular Diseases: Therapeutic Effect of Barley. – DIET FACTOR (Journal of Nutritional and Food Sciences) 3(2).

[31] Nowak, R., Szczepanek, M., Kobus-Cisowska, J., Stuper-Szablewska, K., Dziedziński, M., Błaszczyk, K. (2023): Profile of phenolic compounds and antioxidant activity of organically and conventionally grown black-grain barley genotypes treated with biostimulant. – Plos One 18: e0288428.

[32] Oluwajuyitan, T. D., Kadiri, O., Origbemisoye, B. A., Fagbohun, O. F., Ukejeh, R. N., Popoola, O., Olawoye, B. (2021): Cereal Grain: A Vehicle for Improved Healthy Living. – Cereal Grains-Volume 1. IntechOpen.

[33] Raj, R., Shams, R., Pandey, V. K., Dash, K. K., Singh, P., Bashir, O. (2023): Barley phytochemicals and health promoting benefits: A comprehensive review. – Journal of Agriculture and Food Research 14: 100677.

[34] Rani, H., Bhardwaj, R. D., Sen, R., Kumar, D., Bhardwaj, P., Kaur, S., Grewal, S. K. (2024): Deciphering the potential of diverse barley genotypes for improving the malt quality. – Journal of Stored Products Research 105: 102247.

[35] Rawat, M., Varshney, A., Rai, M., Chikara, A., Pohty, A. L., Joshi, A., Binjola, A., Singh, C. P., Rawat, K., Rather, M. A. (2023): A comprehensive review on nutraceutical potential of underutilized cereals and cereal-based products. – Journal of Agriculture and Food Research 12: 100619.

[36] Shvachko, N. A., Loskutov, I. G., Semilet, T. V., Popov, V. S., Kovaleva, O. N., Konarev, A. V. (2021): Bioactive components in oat and barley grain as a promising breeding trend for functional food production. – Molecules 26: 2260.

[37] Steel, R. G., Torrie, J. H. (1986): Principles procedures of statistics: a biometrical approach. – McGraw-Hill.

[38] Teoh, Y. J., Cheng, W. H., Wider, W., Krishnan, K., Yap, C. K., Chen, Q., Jiang, L., Lei, T. M. T., Tanee, T., Sudmoon, R., Lee, S. Y. (2024): Heavy metal accumulation in paddy plants and health risks: Insights from southern peninsular Malaysia and global research trends. – Asian Journal of Agriculture and Biology 2: 2023076.

[39] Thabet, S. G., Alomari, D. Z., Brinch-Pedersen, H., Alqudah, A. M. (2022): Genetic analysis toward more nutritious barley grains for a food secure world. – Botanical Studies 63: 6.

[40] Victoria, O., Idorenyin, U., Asana, M., Jia, L., Shuoshuo, L., Yang, S., Okoi, I. M., Ping, A., Egrinya, E. A. (2023): Seed treatment with 24-epibrassinolide improves wheat germination under salinity stress. – Asian Journal of Agriculture and Biology 3: 2022076.

[41] Wijekoon, C., Netticadan, T., Sabra, A., Yu, L., Kodikara, C., Badea, A. (2022): Analyses of fatty acids, proteins, ascorbic acid, bioactive phenolic compounds and antioxidant activity of Canadian barley cultivars and elite germplasm. – Molecules 27: 7852.

[42] Xiong, Y., Zhang, P., Warner, R. D., Shen, S., Fang, Z. (2022): Cereal grain-based functional beverages: From cereal grain bioactive phytochemicals to beverage processing technologies, health benefits and product features. – Critical Reviews in Food Science and Nutrition 62: 2404-2431.

[43] Xue, W., Gianinetti, A., Wang, R., Zhan, Z., Yan, J., Jiang, Y., Fahima, T., Zhao, G., Cheng, J. (2016): Characterizing barley seed macro-and micro-nutrients under multiple environmental conditions. – Cereal Research Communications 44: 639-649.

[44] Yang, T., Zeng, Y.-W., Pu, X.-Y., Du, J., Yang, S.-M. (2013): Correlation analysis of functional components of barley grain. – In: Zhang, G., Li, C., Liu, X. (eds.) Advance in Barley Sciences. Springer, Dordrecht, pp. 199-207.

[45] Yang, T., Zeng, Y., Du, J., Yang, S., Pu, X. (2019): Genetic analysis of four main flavonoids in barley grain. – Bangladesh Journal of Botany 48: 231-237.

[46] Yiblet, Y., Misganaw, W., Adamu, E. (2024): Nutritional and Functional Composition of Barley Varieties from Legambo District, Ethiopia. – The Scientific World Journal 2024: 1367540.

[47] Zeng, Y., Pu, X., Du, J., Yang, X., Li, X., Mandal, M. S. N., Yang, T., Yang, J. (2020): Molecular mechanism of functional ingredients in barley to combat human chronic diseases. – Oxidative Medicine and Cellular Longevity 2020: 3836172.