Sustainable Food Proteins

WILEY

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ORIGINAL ARTICLE OPEN ACCESS

Characterization of Protein Profiles and Their Role in Malting Quality of Commercial Barley Genotypes (*Hordeum vulgare*)

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Received: 12 May 2025 | **Revised:** 24 October 2025 | **Accepted:** 31 October 2025

Funding: This work was supported by Universidad Nacional de Rio Negro (PI-UNRN 40-C-1063) and Consejo Nacional de Investigaciones Científicas y Técnicas (Grant number: 28720210100475CO).

Keywords: amylolytic enzyme activity | germinative capacity | hordeins | micro-malted

ABSTRACT

In Argentina, the cultivation of malting barley (*Hordeum vulgare* L.) has expanded significantly, driven by the increasing demand of the brewing industry for barley malt. However, the predominance of the Andreia cultivar has reduced genetic diversity, increasing its vulnerability to environmental fluctuations and limiting the selection of new genotypes with desirable malting traits. Based on the hypothesis that significant biochemical variability exists among the commercial genotypes currently available in Argentina, this study provides the first comprehensive assessment integrating germination performance, protein content, hordein composition, and amylolytic enzyme activity in these genotypes. Twelve malting barley genotypes were evaluated under uniform growing conditions, and biochemical analyses were conducted on micromalted grains. Gel Electrophoresis revealed polymorphisms mainly in B- and C-hordeins, supporting genetic differentiation among cultivars. The total protein content ranged from 9.5% to 13.2%, whereas soluble hordeins varied from 5.8% to 12.9% of the total protein. α-Amylase activity values ≥150 U/g were observed in all cultivars evaluated, except for Sinfonía. However, amylolytic activity, which is related to diastatic power, was higher in genotypes such as Andreia, Alhue, Aliciana, and Jennifer. Correlations between protein content, enzyme activity, and hordein profiles indicated genotype-dependent biochemical patterns in the barley grains. Overall, this study highlights the existence of relevant biochemical diversity among Argentine commercial genotypes, providing valuable information for breeding programs aimed at improving malting quality and promoting productive diversification in the national barley industry.

1 | Introduction

Barley (*Hordeum vulgare* L.) is a cereal of global importance, not only for animal and human consumption but also as a key raw material for the brewing industry. In Argentina, the cultivation of malting barley has expanded significantly in response to growing demand, positioning the country in tenth place in the world market (FAO 2024). However, this expansion has been accompanied by the marked predominance of a few cultivars, with "Andreia" being the most widely grown one (Gonzalez et al. 2024). Over the last century, genetic improvement programs have traditionally favored traits related to yield, leading to a loss of genetic diversity and increased crop susceptibility. This compromises production stability and the resilience of the agro-industrial sector in the face of climate change and market fluctuations (Bélanger and Pilling 2019). Genetic diversity is essential for crop adaptability and the development of new and improved varieties (Simón and Golik 2022). The rise of craft beer has driven the demand for malting barley with quality characteristics that allow the production of beers with unique flavor profiles (Borgna 2019; García Paz et al. 2024; Aguirre Tenesaca and Illescas Yunga 2024). In barley, genetic diversity can be assessed through various methodologies such as agromorphological traits, biochemical markers, and molecular markers (Atanassov et al. 2001; Dido et al. 2020; Simón and Golik 2022; Khera et al. 2025). Although agromorphological markers may have limitations in their specificity and be affected by genotype-environment interactions, biochemical markers, such as hordeins, have proven to be valuable tools. Hordeins exhibit high variation between genotypes, which has been used to identify cultivars, analyze genetic diversity, and study phylogenetic origin (Atanassov et al. 2001; Salgado-Albarrán et al. 2015; López Alvarado 2019). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), allows the genetic structure of germplasm to be described through the separation of these proteins, thereby enabling the differentiation of cultivars (Salgado-Albarrán et al. 2015; López Alvarado 2019). This is possible because each variety has a characteristic hordein migration pattern and, unlike other traits, is less influenced by the environment, as they are tolerant to mutations (Fantahun et al. 2023). Exploring the genetic diversity of barley cultivars available in the Argentine market could allow the identification of genotypes with specific quality attributes for various industrial applications. In the context of malting barley, quality traits are crucial and intrinsically linked to the biochemical composition of the grains (Izydorczyk and Edney 2017; Khera et al. 2025). Barley grain is mainly composed of the embryo, endosperm, and pericarp, the latter being moderately lignified. The endosperm, which accounts for nearly 80% of the dry weight, consists primarily of starch embedded in a protein matrix that is easily degradable, thereby enhancing fermentability. In terms of proteins, barley is characterized by a high proportion of hordeins, which represent more than half of its total protein content (Martínez 2020). From a biochemical standpoint, barley seeds possess a complex composition that defines their technological behavior during malting and brewing. The endosperm mainly contains starch (32.4%–60.3%), proteins (8.6%–17.4%), amylose (13.3%–19.3%), and nonstarch polysaccharides such as β-glucans (1.31%–6.06%), which influence water uptake, enzyme activity, and wort viscosity, whereas minor components such as lipids (2%–3%) and minerals (~2%) contribute to foam stability and fermentation performance (Lukinac and Jukić 2022; Khera et al. 2025). Storage proteins, primarily hordeins, globulins, and albumins, fulfill structural and metabolic functions, and their degradation during germination releases amino nitrogen compounds that are essential for yeast growth. The proportion and interaction of these constituents ultimately determine malt modification, yield, and the sensory quality of the final beer (Izydorczyk and Edney 2017). Among the most relevant quality parameters are total protein content, hordein composition, and the activity of hydrolytic enzymes, particularly α- and β-amylases (Howard et al. 1996; Tanner et al. 2019; Bahmani et al. 2024). Protein levels are decisive because they affect both malt yield and brewing performance: excessive protein can reduce extract efficiency and beer quality, while insufficient levels may limit enzymatic activity (Vinje et al. 2019). Simultaneously, the composition of hordein fractions determines protein solubility and the degree of endosperm modification, which directly affects starch accessibility. Complementarily, α- and β-amylases play central roles in starch hydrolysis, ensuring efficient wort production. Together, these parameters define the balance between extract potential, enzymatic power, and fermentability, which are critical for malting quality and beer quality. The uniqueness of this study lies in providing the first comprehensive assessment of biochemical diversity, including the hordein profile and amylolytic enzyme activity, in a representative set of commercial malting barley genotypes grown in Argentina. Unlike previous studies, this research focuses on cultivars currently available to producers, which has direct and practical relevance for the diversification of the production matrix and the selection of genotypes with desirable quality profiles for the national beer industry. Based on the limited diversity reported in current production and the inherent variability of barley populations, the hypothesis of this study is that there is significant biochemical diversity among the commercial barley cultivars available in Argentina. These differences correlate with variations in key malting quality parameters, which could offer viable alternatives for the brewing industry. Therefore, the objective of this study was (i) to identify the quality-related traits (protein content, hordeins, and activity of amylolytic enzymes) of 12 commercial malting barley genotypes and (ii) to determine the correlation between the different traits to provide information for malting barley improvement programs in Argentina.

2 | Materials and Methods

2.1 | Genotypes and Sample Preparation

Twelve commercial barley genotypes: Yanara (1), Fátima (2), Militza (3), Danielle (4), Montoya (5), Charles (6), Alhue (7), Sinfonía (8), Overture (9), Aliciana (10), Jennifer (11), and Andreia (12). Samples of dried barley grains were supplied by the National Network of Brewery Barley of the National Institute of Agricultural Technology (INTA, Argentina). These genotypes were part of a network of comparative yield trials conducted in 2017 in Bordenave (Argentina). The materials were grown under the same experimental conditions in a completely randomized block design with three replicates per treatment. Barley grains from each genotype were ground using a high-speed blade mill (Model Fw-100, Zelian, Argentina). The resulting flour was dried at 50°C for 22 h and stored at −20°C for later use.

2.2 | Germination Process

Barley seeds were surface-sterilized (70% ethanol, 10 min; 20% sodium hypochlorite, 20 min), rinsed, and germinated in quadruplicate (25 seeds/dish) at 15°C in the dark. Germination was defined as radicle emergence ≥ 0.5 mm (Bewley and Black 2013).

2.2.1 | Estimation of Germination Parameters

To observe the seed germination rates, the number of germinated seeds was recorded every 4 h. The following parameters were estimated: Germination energy (GE): Percentage of accumulated daily germination when the germination rate was highest (Pece et al. 2010). Germination Capacity (GC): Percentage of total germination at the end of the test. Average Germination

Time (AGT): Average number of hours used for germination. It measures the speed and dispersion of the process (Ranal and Garcia de Santana 2006).

$$AGT = ((X1.\,h4) + (X2.\,h8) + \ \ldots\ + (X9.\,h72))\,/\,\text{total seeds}.$$

where X1, X2, and X9 are the seeds at 4 h, h4, h8,... h72.

2.2.2 | Micromalting Process of Barley Grains

Twelve barley cultivars underwent malting and germination processes, following Passarella et al. (2003). Briefly, 10 g grain samples were subjected to alternating 4–5 h hydration and 19–20 h drying cycles (15°C, dark) over two days, followed by a 4-day germination period with 4 h interval germination counts.

2.3 | Evaluation of Protein Fractions

2.3.1 | Total Protein

The total protein content (%) was determined in duplicate for each barley cultivar using the Kjeldahl method (Association of Official Analytical Communities 1990), with a nitrogen-to-protein conversion factor of 6.25.

2.3.2 | Hordein Extraction

Flour from unmalted cultivars underwent alcoholic extraction, as described by Howard et al. (1996). Samples were stirred in an extraction buffer (55% propanol, 1% acetic acid, 2% beta-mercaptoethanol) at 60°C for 1 h, centrifuged, and the supernatant was collected for analysis.

2.3.3 | Extraction of Amylolytic Enzymes in Barley Grains

Amylolytic enzymes were extracted from unmalted and malted seed flours using the modified method described by Howard et al. (1996). Prior to extraction, malted seeds were dried at 50°C for 24 h in a forced-air oven until a constant weight was reached and then ground using a Zelian FW-100 high-speed blade mill (Argentina) to obtain flour. Next, 0.1 g of flour was suspended in 1 mL of extraction buffer (15 mM potassium phosphate, pH 8.0; 5 mM β-mercaptoethanol). The mixture was stirred for 1 h at room temperature to facilitate enzyme extraction. The samples were centrifuged at 10,000 rpm for 5 min, and the resulting supernatant, containing the extracted enzymes, was carefully collected.

2.4 | Physicochemical Characterization of Enzyme Fractions

2.4.1 | Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The hordein fractions obtained were characterized on 12% polyacrylamide gels using the SDS-PAGE method of Laemmli (1970). After electrophoresis, the gels were fixed, stained with 0.01% Coomassie Brilliant Blue, and destained in distilled water for 24 h.

2.4.2 | Determination of Soluble Hordeins

The soluble hordein content was determined using the Bradford (1976), using bovine serum albumin as a standard (0–20 μg). All assays were performed in triplicate, and soluble proteins were expressed in mg/mL.

2.5 | Genetic Clustering Analysis

The study of genetic variability was performed by creating a binary matrix of the absence/presence of hordein electrophoretic bands obtained in 4.1. A dendrogram was constructed using hierarchical clustering analysis with the Gower coefficient as the dissimilarity metric and the centroid method as the linkage rule. Statistical analysis was performed using the Multivariate Statistical Package Data 3.1 (MVSP 3.1) using the Nei-Li coefficient (Nei and Li 1979).

2.6 | Evaluation of Biochemical Parameters for Malting Quality

2.6.1 | Determination of α-Amylase Activity

α-Amylase activity was measured according to Pérez et al. (2018), using Amilokit (Wiener Group, Argentina) following the manufacturer's instructions. Absorbance was measured at 640 nm using a spectrophotometer, and all assays were performed in triplicate. The activity was defined as U/mL, according to the manufacturer's specifications.

2.6.2 | Quantification of Total Amylolytic Activity

2.6.2.1 | Enzymatic Hydrolysis of Extracts. To determine amylolytic activity, the diluted extracts (100-fold) were incubated with a starch solution (0.17% in 10 mM citrate buffer pH 5.5, 3.4 mM $CaCl_2$) for 10 min at 50°C. A total of 0.5 mL of 0.5 M NaOH was used to terminate the reaction (De Schepper et al. 2021).

2.6.2.2 | Quantification of Reducing Sugars. The reducing sugars released after enzymatic starch hydrolysis were determined using the Somogyi (1945). All assays were performed in triplicate. Absorbance was measured at 520 nm, with a 1% (w/v) glucose standard used for a calibration curve performed in triplicate. Amylolytic enzyme activity was expressed as units of activity per microgram of protein (U/μg protein). One unit (U) of enzyme activity was defined as the amount of enzyme that catalyzes the formation of 1 mmol of reducing sugars per minute, under the assay conditions.

2.7 | Statistical Analysis of Data

Data were analyzed using one-way analysis of variance (ANOVA), and Fisher's 5% significance level and Least

Significant Difference (LSD) were used to determine the differences between the means. Principal component analysis (PCA) was also performed to identify the properties underlying the differences between cultivars associated with the biochemical attributes of malting quality (soluble hordeins, α-amylase activity, amylolytic activity, GC and AGT). The variables were projected onto a biplot graph representing the first two principal components (PC1 and PC2). This approach allowed the visualization of relationships between variables and the distribution of varieties in a two-dimensional space, facilitating the identification of clustering patterns and the variables that contribute most significantly to the variance. All statistical analyses were performed using the INFOSTAT statistical program, student version (Di Rienzo 2009) and for the principal component analysis, the free software PAST 4.17c was used.

3 | Results and Discussion

3.1 | Determination of Total Protein Content in Barley Grains

For the commercialization of barley in the brewing industry, key grain characteristics, such as total protein content (quantified as total nitrogen) and grain size (proportional to starch content), are crucial indicators of malting quality (Table 1). In our study, the total protein content was significantly different ($p<0.05$) among the 12 barley cultivars. However, all cultivars demonstrated acceptable values for marketing, as established by Argentine regulations, which specify a minimum protein content of 9.5% and a maximum of 13% (Bolsa de Comercio de Rosario 2013). Protein content in malting barley is a critical quality parameter in the brewing industry. The ideal range is typically between 10% and 12% as deviations can negatively impact the brewing process and final product quality. Low protein content can limit yeast growth during fermentation due to insufficient nitrogen, while high protein levels can lead to undesirable results, such as decreased malt extract yield and various processing problems, including filtration issues and excessive color development during wort boiling (Véliz 2017). Furthermore, it contributes to the formation of chill haze, which is the precipitation of protein complexes in cold beer (Holopainen et al. 2005). These challenges are significant, as they lead to the rejection of barley for malting when the protein levels fall outside the acceptable range. In addition, the characterization of these reserve proteins could open new opportunities to valorize barley, expanding its applications beyond brewing toward the development of innovative foods and functional ingredients, potentially utilizing spent grain as a valuable raw material (Fărcaș et al. 2021).

3.1.1 | Solubilization of Hordeins

The main protein fractions found in barley grains are hordeins, which are soluble in alcohol–water mixtures. Given their high proline and glutamine content, hordeins are classified as prolamins (Hernández-Espinosa et al. 2015). The soluble prolamins are listed in Table 1 and showed significantly different ($p<0.05$). Among the cultivars, Yanara, Fatima and Alhue exhibited lower solubilized hordein content (5.8%, 8%, and 8.7%, respectively) compared to the other cultivars, which ranged from 9.9% to 12.9%. Conversely, the Jennifer and Andreia cultivars showed the highest levels of soluble hordeins (12.9 and 11.2%, respectively). Although a higher protein content indicates an increase in reserve proteins, particularly hordeins, the solubility of these proteins is influenced by the nature of their fractions (Roustan et al. 2018). Studies have indicated that a higher proportion of ϒ- and B-hordeins, which are rich in cysteine and sulfhydryl groups, facilitates intra- and interchain disulfide bond formation, leading to protein aggregation and reduced solubility. In contrast, C-hordeins (55–70 kDa), which are smaller than D-hordeins, comprise 10%–20% of the total proteins. Owing to the absence of cysteine residues, they do not form complexes with other proteins (Martínez 2020). Based on our results, we conclude that the

TABLE 1 | Biochemical and physical characterization of malting barley (*Hordeum vulgare*) genotypes: total protein content, seed size, and soluble hordein fraction.

Commercial lines	Total protein (g/100 g flour)	Seed size (g/seed)	Soluble hordeins (g/100 g Total protein)
Yanara	10.98 ± 0.88d	0.046 ± 0.03 c	5.8 ± 0.17e
Fátima	11.21 ± 0.56b	0.045 ± 0.02 c	8.0 ± 0.19d
Militza	10.96 ± 0.55e	0.047 ± 0.02 bc	9.9 ± 1.2c
Danielle	10.85 ± 0.87f	0.032 ± 0.03 d	10.2 ± 0.84c
Montoya	10.54 ± 0.53 g	0.044 ± 0.01 c	10.9 ± 0.02bc
Charles	11.52 ± 0.58a	0.034 ± 0.03 d	10 ± 0.74bc
Alhue	10.32 ± 0.52i	0.051 ± 0.01 a	8.7 ± 0.68d
Sinfonía	10.21 ± 0.51j	0.050 ± 0.02 ab	11.1 ± 0.15bc
Overture	10.08 ± 0.81 k	0.044 ± 0.02 c	10.5 ± 0.72c
Aliciana	10.50 ± 0.52 h	0.044 ± 0.01 c	10.2 ± 0.71c
Jennifer	11.05 ± 0.88e	0.046 ± 0.02 c	12.9 ± 0.25a
Andreia	11.22 ± 0.90b	0.047 ± 0.02 bc	11.2 ± 0.4b

Note: Data are expressed as mean ± standard deviation. Different letters in the same column indicate a significant difference ($p<0.05$) according to Fisher's LSD test.

Jennifer and Andreia cultivars could be characterized by a reduced proportion of ɤ and B hordeins, and a higher proportion of C hordeins. In contrast, the Yanara, Fatima, and Alhue cultivars could have a higher proportion of ɤ and B hordeins, due to their lower soluble hordein content. So results suggest that a higher insoluble hordein content could constitute a technological limitation, as it reduces starch degradation and the consequent release of fermentable sugars, negatively affecting malt extract yield. The malting quality of a barley genotype is primarily determined by the efficiency of storage protein hydrolysis into soluble fractions, a process catalyzed by endoproteinases (Hayes et al. 2003). In this sense, the partial conversion of insoluble native proteins into soluble fractions not only increases the yield but also impacts key parameters of beer quality, such as foam stability and the final sensory profile (Steiner et al. 2011).

3.2 | Physicochemical Characterization of Hordeins by Electrophoresis

Hordein is the main storage protein in barley and exhibits a high degree of polymorphism owing to its multigenic origin. Figure 1 presents the results of the protein profile analyses, showing 5–9 protein bands per cultivar, with molecular weights ranging from 20 to 100 kDa. The Yanara, Fatima, and Alhue presented a greater number of ɤ and B hordein bands (36–44 kDa) than the other cultivars analyzed. This observation is consistent with the reduced solubility data shown in Table 1. In contrast, the Jennifer and Andreia had fewer ɤ and B bands of hordein and higher solubility than the other cultivars. Hordein B polymorphisms were evident among the genotypes analyzed. Cultivars Yanara, Fátima, Militza, Montoya, Alhue, Aliciana, and Jennifer showed four bands each, while Sinfonía showed two bands. In the remaining samples (Danielle, Charles, Overture, and Andreia), three bands ranging from 36 to 48 kDa were observed, aligning with previous studies (Leistrumaitė and Paplauskienė 2007). Jennifer, Aliciana, Sinfonía and Andreia displayed a distinct C-hordein band (55–70 kDa). The observed hordein polymorphism serves as a genotypic marker and has a direct impact on malting and brewing quality. Specifically, the variable abundance and composition of hordeins B and C influence key industrial parameters, affecting starch conversion efficiency, malt extract yield, and ultimately the essential characteristics of the final beer, such as clarity and foam stability (Holopainen et al. 2005). It has been reported that the degradation of hordeins during malting varies depending on their type. For example, fractions D and C undergo greater enzymatic degradation, whereas γ and B hordeins are more resistant to the process (Flodrová et al. 2012). The resistance of γ and B hordeins, together with other nonprolamin proteins, plays a crucial role in the formation of turbidity in beer (Jin et al. 2012). D-hordeins (96–100 kDa) and ɤ-hordeins (~20 kDa) were consistently present in all samples. The distinct hordein profiles identified among cultivars reflect genetic polymorphisms that could serve as reliable markers for genetic clustering, offering practical applications in barley breeding programs aimed at enhancing malt quality. Because all samples were cultivated under the same environmental conditions, the observed electrophoretic variability can be largely attributed to genetic differences, thereby minimizing the confounding effects of the environment. This study constitutes the first characterization of hordein pattern variability in commercial malting barley varieties from Argentina, providing a valuable baseline for future breeding strategies. However, to establish the stability and heritability of these protein markers, it is essential to validate their expression across multiple environments and growing seasons to ensure their effectiveness as selection tools in breeding programs.

3.3 | Clustering of Genotypes Based on Hordeins Profiles

Hordeins show great intergenotypic variation and are still used as markers for cultivar identification and genetic diversity studies (Fantahun et al. 2023). In this study, similarity analysis of the electrophoretic profiles obtained (Figure 1) produced a dendrogram that visually represented the grouping of the analyzed cultivars (Figure 2). With 65% similarity, the cultivars were grouped into six clusters. Fatima and Militza, and Aliciana and

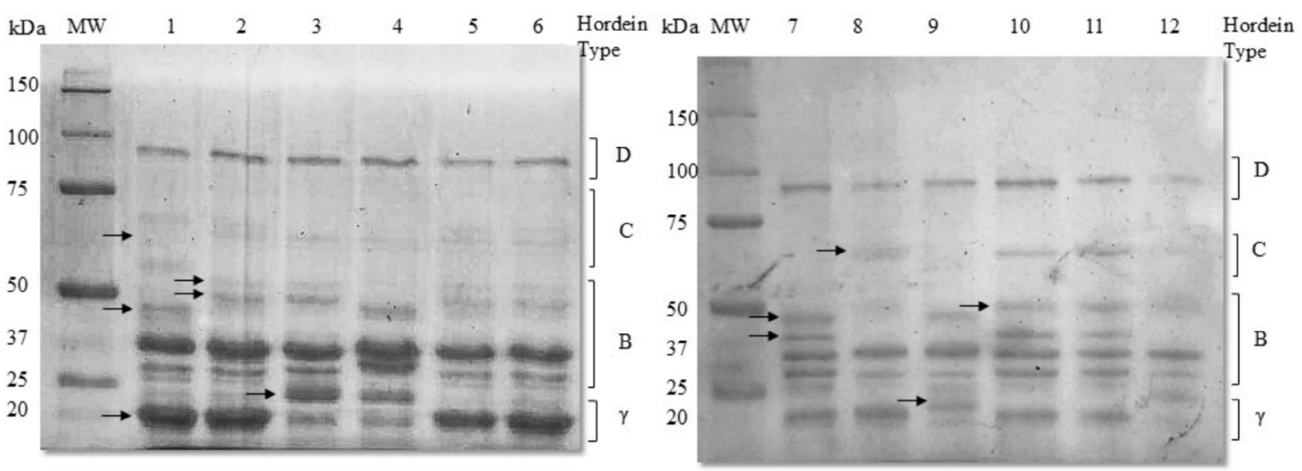

FIGURE 1 | Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of hordeins extracted from single grains of 12 *Hordeum vulgare* genotypes: (1) Yanara, (2) Fatima, (3) Militza, (4) Danielle, (5) Montoya, (6) Charles, (7) Alhue, (8) Sinfonía, (9) Overture, (10) Aliciana, (11) Jennifer, and (12) Andreia. Protein bands are classified as γ-hordeins (~20 kDa), B-hordeins (~30–45 kDa), C-hordeins (~48–65 kDa), and D-hordeins (~90 kDa). Black arrows highlight polymorphic bands that reveal inter-cultivar variation.

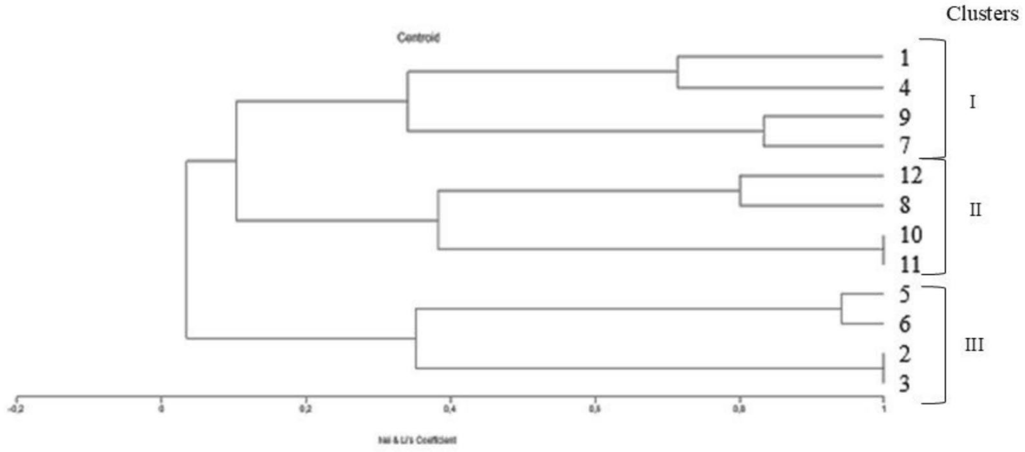

FIGURE 2 | Dendrogram of 12 malting barley genotypes (*Hordeum vulgare*) obtained from the cluster analysis of hordein electrophoretic profiles (SDS-PAGE), using the Nei and Li similarity coefficient and the centroid method. Three main groups were identified: Cluster I (Yanara, Danielle, Overture, Alhue), Cluster II (Andreia, Sinfonía, Aliciana, Jennifer), and Cluster III (Montoya, Charles, Fatima, Militza).

Jennifer showed 100% similarity. Montoya and Charles shared 94.1% similarity, clustering with Fatima and Militza (0.35 distance). Sinfonia and Andreia clustered with 80% similarity, whereas Alhue and Overture clustered with 83.3% similarity. Yanara and Danielle clustered with 71.4% similarity, with a distance of 0.35 from Overture and Alhue. Three groups of four cultivars were identified with 35% similarity, suggesting a possible common genetic origin. Cultivars Fátima, Militza, Montoya, and Charles showed a distinct genetic origin, although phylogenetic sequencing is required to confirm this. Fatima and Militza exhibited shared hordein bands. Similarly, Montoya and Charles displayed comparable patterns, differing only by a 30 kDa band corresponding to hordein B. Jennifer and Aliciana shared bands with those of Sinfonia and Andreia, but differed by 66 kDa (hordein C) and 48/40 kDa (hordein B) bands. Overture and Alhue were similar to Yanara and Danielle. These differences underscore the polymorphism of hordein B, as previously reported. In summary, SDS-PAGE analysis of hordeins was effective in characterizing and differentiating barley cultivars, highlighting the value of seed protein polymorphisms as tools for cultivar identification and discrimination in barley. Clustering analysis confirmed that Andreia, despite being the most widely used malting barley genotype, shares a relatively high degree of similarity with Sinfonia (80%), indicating a narrower genetic base within this group. This result is particularly relevant given the widespread use of Andreia in Argentina, as it highlights the potential risk of reduced genetic diversity in commercial malting barleys. Therefore, integrating underutilized genotypes with divergent hordein patterns into breeding strategies may contribute to enhancing resilience, maintaining malting quality, and ensuring the long-term sustainability of barley production in Argentina.

3.4 | Germination Parameters and Their Effect on Malting

Malting involves the controlled germination of barley seeds at 15°C, followed by drying at temperatures between 55°C and 82°C (Giménez 2017). This essential brewing process is closely influenced by barley grain germination parameters. Table 2 summarizes the results obtained for the germination parameters of the 12 cultivars, which were significantly different ($p < 0.05$). Germination capacity (GC), assessed after 3 days of germination (the standard time frame for malting), revealed that most cultivars exhibited values exceeding 95%–98%. These values align with the minimum and optimal percentages required for the commercialization of malting barley (Cámara Arbitral de Cereales de Buenos Aires, Resolución n° 27/2013). However, two cultivars, Alhue and Aliciana, showed GC values below the commercialization standards, with 93% and 81%, respectively. In contrast, germination energy (GE) serves as an indirect indicator of seed vigor. As malting requires rapid initiation of germination, optimally malted barley

TABLE 2 | Germination performance of malting barley (*Hordeum vulgare*) genotypes: germination capacity (GC) and average germination time (AGT).

Commercial lines	GC[a] (%)	AGT[b] (h)
Yanara	95 ± 0.50e	20.60 ± 1.87f
Fátima	98 ± 1.00bc	19.68 ± 0.32h
Militza	100 ± 0.00a	22.80 ± 0.36a
Danielle	99 ± 0.50ab	20.80 ± 0.22e
Montoya	95 ± 1.26e	21.60 ± 0.41d
Charles	98 ± 0.56bc	22.56 ± 0.15b
Alhue	93 ± 0.50f	19.72 ± 0.28h
Sinfonía	96 ± 0.82de	19.44 ± 0.11i
Overture	98 ± 0.56bc	21.92 ± 0.39c
Aliciana	81 ± 1.71g	20.76 ± 0.63e
Jennifer	95 ± 0.96e	19.04 ± 0.27j
Andreína	97 ± 0.96ab	20.08 ± 0.24g

Note: Data are expressed as mean ± standard deviation. Different letters in the same column indicate a significant difference ($p < 0.05$) according to Fisher's LSD test.
[a]Germinative capacity.
[b]Average germination time.

must exhibit high GE (Celus et al. 2006). The Andreia cultivar demonstrated the highest GE, with 32% germination within the first 16h of soaking. The Danielle, Yanara, and Fátima cultivars reached GE at 20h, with germination rates of 34%, 38%, and 46%, respectively. The remaining cultivars reached their maximum germination rates after 24h. This analysis of GE holds particular importance for malting, as it helps predict the proportion of seeds that will germinate rapidly, thus minimizing the risk of pathogen attack during the process and ensuring efficiency and quality in malting (Rooney et al. 2023). The average germination time (AGT) for the Andreia cultivar was 20.08h, which was within the range observed for other cultivars. Although the Jennifer cultivar exhibited the lowest AGT at 19.04h, this parameter alone was insufficient to determine the overall suitability of barley for malting. The joint evaluation of GC, GE, and AGT identified the Andreia and Jennifer cultivars as those with the most favorable germination characteristics for malting barley production. Their superior performance could be associated with a higher soluble hordein content, which is an indicator of high proteolytic activity (Nye-Wood and Colgrave 2024). This factor not only promotes the solubilization of reserve proteins, but also increases the availability of starch for germination. Together, these properties promote more efficient endosperm modification, a key aspect in ensuring both quality and yield in malt extract production.

3.5 | Effect of Micromalting Process on α-Amylase Activity

Germination is a biological process that triggers the synthesis of new proteins and increases the solubility of existing proteins (Bahmani et al. 2024). In the malting process of barley for brewing, proteins play a crucial role in influencing beer characteristics, such as mouthfeel, body, foam stability, and haze formation (Ferreyra 2014). The micromalting process led to a 1.85-fold increase that showed significantly different ($p < 0.05$) in eight cultivars, while Danielle, Montoya, Charles, and Sinfonía showed no differences compared to unmalted grains (data not shown). During germination, enzymes such as α- and β-amylase and endoproteinases play key roles in hydrolyzing starch granule-protein complexes and solubilizing proteins. Mobilization and hydrolysis of storage protein reserves result in a heterogeneous mixture of soluble nitrogen compounds, including enzymes, small peptides, and free amino nitrogen, which are valuable nitrogen sources for yeast during malt production (Roustan et al. 2018). Besides, these proteins have a significant influence on the characteristics of beer, as an adequate amount is needed to stabilize the foam, but without excess that could cause turbidity or make the process more expensive. The α-amylase content of the germinated barley grains is presented in Figure 3. The cultivars Militza, Alhue, Overture, and Aliciana exhibited higher enzyme levels, whereas Fátima, Sinfonía, and Andreia showed α-amylase concentrations below 50%. α-Amylase activity represents a critical quality parameter in barley breeding, and it is well established that enzyme levels of ≥150U/g are required to satisfy the standards of the malting industry (Fox et al. 2003). In this regard, all the cultivars evaluated were within this range, with the exception of Sinfonia. The key amylolytic enzymes for starch degradation in barley malt are α- and β-amylase, α-glucosidase, and a limited amount of dextrinase and the combined activity of these enzymes is known as diastatic power (DP) (Collins et al. 2021). Therefore, the combined activity of amylolytic enzymes must be evaluated to determine brewing quality.

3.6 | Evaluation of Amylolytic Activities

To corroborate the synergistic activity of amylolytic enzymes present in micromalted and nonmicromalted grains, in vitro hydrolytic activity was evaluated using a starch solution as a substrate. As shown in Figure 4, nonmicromalted grains show low starch hydrolytic capacity, likely due to the absence of the proteins that can influence the brewing quality of barley such

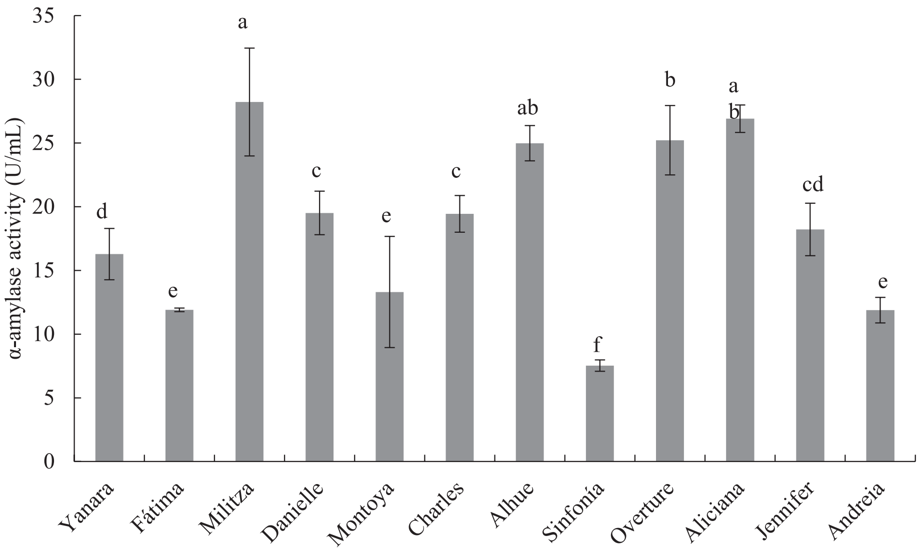

FIGURE 3 | α-Amylase activity in 12 malting barley (*Hordeum vulgare*) genotypes. Data represent the mean ± standard deviation (SD) of three independent experiments, expressed in U/mL. Different letters denote significant differences between cultivars ($p < 0.05$, Fisher's LSD test).

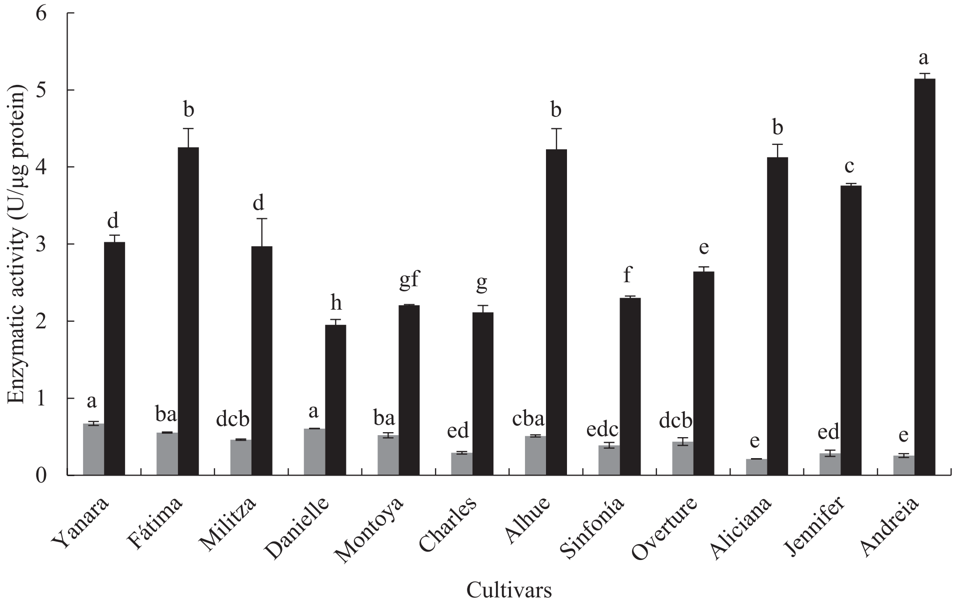

FIGURE 4 | Comparative amylolytic activity in nonmicromalted (■) and micromalted (■) grains from 12 malting barley (*Hordeum vulgare*) genotypes. Results are expressed as the mean ± SD of three independent experiments in units per microgram of protein (U/µg protein). Different letters indicate significant differences between treatments ($p < 0.05$, Fisher's LSD test). The assay reflects the hydrolytic capacity of protein extracts to degrade starch, evidencing changes induced by micromalting.

as β-amylase and β-glucosidase (Strouhalova et al. 2018). After the micromalted process, the cultivars Fátima, Alhue, Alician, Jennifer, and Andreia produced the highest reducing sugar concentrations where significant differences were observed ($p < 0.05$). These could include glucose, fructose, galactose, lactose, and maltose, which are products of starch hydrolysis mediated by amylolytic enzymes (Pereira et al. 2018). The Andreia cultivar demonstrated the highest activity, underscoring its potential for achieving an elevated extract yield, an essential economic trait in brewing. Higher fermentable sugar yields not only improve a cultivar's competitiveness in international markets by reducing the raw material requirements to produce the same volume of alcohol (Paredes Espinoza 2017) but also ensure the rapid availability of sugars for fermentation. Although multiple enzymes are involved in determining malting quality, various studies have shown that β-amylase activity in barley correlates positively and significantly with the total protein content of the grain, particularly with type C hordeins (Bahmani et al. 2024). In summary, the superior hydrolytic capacity of the Andreia, Aliciana, and Jennifer cultivars appears to result from the combined influence of their amylolytic enzyme activity and the similarity of their hordein profiles. The integration of malting quality biochemical parameters with the genetic clustering previously established for these cultivars supports the evidence of a shared genetic origin at both phenotypic and enzymatic levels, thereby reinforcing their malting potential. Moreover, this information provides a valuable framework for diversifying the range of cultivars used in malting, which is particularly relevant given the current predominance of Andreia in commercial production. Principal component analysis (PCA) was applied to evaluate the variability in 12 malting barley cultivars, considering five malt quality variables: soluble hordeins, alpha-amylases, amylolytic activity, average germination time (AGT), and germinative capacity (GC) (Figure 5). The first two principal components (PC1 and PC2) explained 64% of the total variance (36% and 28%, respectively). PC1 differentiated the cultivars mainly by their AGT (0.89) and amylolytic activities (−0.78). PC2 was correlated with α-amylase activity (0.84) and GC (−0.69). Soluble hordeins showed a limited contribution to both components (−0.07 for PC1 and −0.2 for PC2). The PCA biplot grouped the cultivars Andreia, Jennifer, Fátima, and Sinfonía in the left sector, associating them with high amylolytic activity and low AGT, indicating a high malting quality. In contrast, Militza, Charles, and Overture were located in the opposite quadrant, characterized by high AGT and low amylolytic activity.

When considering all evaluated parameters—protein composition, hordein solubility and polymorphism, germination performance, and enzymatic activities—a clear differentiation among genotypes was evident. Integrated biochemical and physiological analyses identified Andreia, Jennifer, and Aliciana as the genotypes with the most balanced profiles, combining high germination energy, elevated amylolytic activity, and adequate protein content within the optimal malting range. Although Aliciana showed a relatively low germination capacity, its high enzymatic efficiency and favorable protein composition suggest that this limitation can be mitigated through optimized malting conditions. The hordein patterns of these genotypes, characterized by a greater proportion of soluble C-hordeins, were associated with enhanced enzyme mobilization and endosperm modification efficiency. In contrast, cultivars such as Militza, Charles, and Overture exhibited lower enzymatic potentials and longer germination times, suggesting reduced malting performance. Overall, the results highlight Jennifer and Aliciana as promising alternatives to Andreia for breeding and production purposes, offering competitive malting quality while contributing to the diversification and genetic resilience of Argentina's barley germplasm.

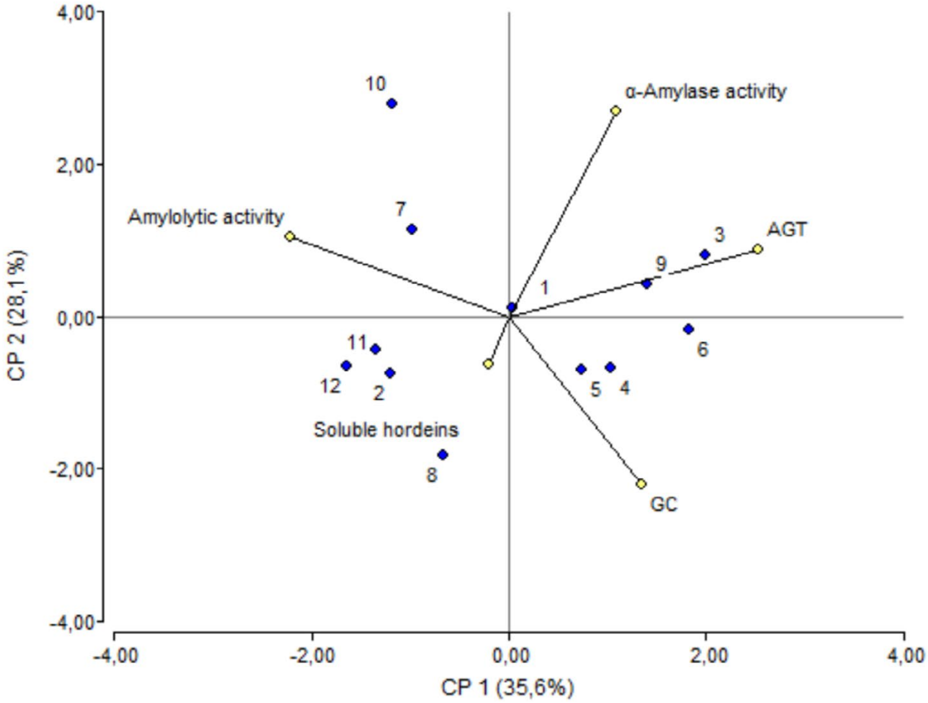

FIGURE 5 | Principal component analysis (PCA) biplot based on biochemical attributes of malting quality for twelve commercial barley (*Hordeum vulgare*) genotypes: (1) Yanara, (2) Fatima, (3) Militza, (4) Danielle, (5) Montoya, (6) Charles, (7) Alhue, (8) Sinfonía, (9) Overture, (10) Aliciana, (11) Jennifer, and (12).

4 | Conclusion

This study provides the first comprehensive biochemical characterization of commercial malting barley genotypes grown in Argentina, revealing a substantial intraspecific diversity in protein composition and enzyme activity. These results highlight how the interaction between storage proteins and hydrolytic enzymes determines endosperm modification and malt quality, providing a biochemical basis for genotypic differentiation. Furthermore, the variability observed in hordein polymorphisms underscores the existence of genotype-dependent biochemical signatures that determine the malting potential. These findings offer valuable information for breeding programs aimed at improving malt quality by selecting genotypes that combine high enzymatic efficiency with an optimal protein profile. Future research should focus on validating these biochemical markers in multiple environments and exploring.

Author Contributions

Fany Zubillaga and Lucrecia Piñuel conceived and designed the study, and wrote the first draft of the manuscript. Eugenia Martínez Diaz carried out the research, Miguel Morón Rivera and Patricia Boeri analyzed the data. All authors contributed to and approved the final draft of the manuscript.

Acknowledgments

The authors would like to thank the INTA Bordenave Agricultural Experiment Station for providing the samples of the different barley genotypes used in this study.

Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

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