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REVIEW

Chemistry of wheat gluten proteins: Quantitative composition

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Abstract

Background and Objectives: Wheat is essential to secure nutrition for the world's population. Its unique processing properties are largely determined by gluten protein content and composition.

Findings: Gluten proteins are subdivided into gluten protein types, α-, γ-, ω1,2-, and ω5-gliadins and high-molecular-weight glutenin subunits and low-molecular-weight glutenin subunits. The overall content and relative proportions of these types vary considerably depending on different genetic and environmental factors and mutual interactions.

Conclusion: This review summarizes the latest developments related to the chemistry of gluten and how species and variety, as well as soil type, weather conditions, atmospheric CO_2 concentration, diseases, and fertilization with nitrogen, sulfur, phosphorus, potassium, and other minerals affect wheat gluten protein composition.

Significance and Novelty: Significant progress has been made to study the effect of different factors on gluten composition. However, comparisons between studies are almost impossible, because of the huge variability in experimental setups, environmental conditions and varieties studied. This calls for a need to develop common guidelines on how to set up experiments, on which parameters to investigate and on which procedure to use to improve comparability and reproducibility of the results.

KEYWORDS
climate change, environmental variability, fertilization, genetics, gliadin, glutenin

1 | INTRODUCTION

The demand for wheat has been steadily increasing over the last five decades and is reflected in the worldwide production quantity of 770 million tons of grains in the year 2019, more than twice the quantity of 300 million tons in the year 1969 (FAOSTAT, 2022). With about 12% of the global population still affected by severe food insecurity and simultaneous population growth, the global food demand is projected to increase by 35%–56% until 2050 according to recent estimates (van Dijk et al., 2021). With more than two-thirds of global wheat production destined for food, wheat will continue to play a critical role in nutrition security, because it is an

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affordable staple food for about 35% of the world population. Wholegrain wheat is a rich source of energy and multiple dietary components essential for human nutrition, with protein being one of the most important (Grote et al., 2021; Wieser et al., 2020). The protein content of wheat grains and flours usually ranges from 7% to 22% (Shewry, 2009), but mostly lies between 10% and 15%. Genetic factors including species and variety explain about one-third of variability (Shewry, 2009), with the other two-thirds being determined by environmental factors such as weather conditions, soil, and atmospheric CO_2 concentration as well as crop management practices including fertilization, tillage, seeding, irrigation and crop rotation, as well as mutual interaction between these factors (Hellemans et al., 2018; Johansson et al., 2013). Flour used for bread making should have more than 10% of protein, corresponding to more than 8% of gluten proteins, because the protein content is known as a major determinant of end-use quality. A sufficient amount of gluten proteins is necessary to develop a continuous proteinaceous network in the dough and to stabilize gas bubbles in leavened doughs and pore walls in baked goods (Wieser et al., 2020).

Apart from the total protein content, the proportions of gluten protein fractions, that is, gliadins and glutenins, and gluten protein types, that is, α-, γ-, ω1,2-, and ω5-gliadins and high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS), vary considerably depending on the different genetic and environmental factors. For details on the qualitative aspects of wheat gluten protein chemistry, please refer to the accompanying review by Wieser et al. (under review). Here, we summarize the latest developments related to the chemistry of wheat gluten proteins and the genetic, environmental and crop management factors determining their quantitative composition.

2 | FACTORS AFFECTING THE QUANTITY OF WHEAT GLUTEN PROTEINS

About 560,000 accessions including wild relatives, landraces, and modern varieties are conserved *ex situ* in wheat germplasm banks (Sansaloni et al., 2020). The availability of the first high-confidence annotated wheat genome reference sequence (The International Wheat Genome Sequencing Consortium [IWGSC], 2018) has facilitated and accelerated high-throughput genotyping to explore wheat diversity and to map evolutionary relationships during the about 10,000 years of wheat domestication and breeding (Pont et al., 2019; Sansaloni et al., 2020). Although proteomics has already provided unprecedented insights into the composition of wheat grain proteins (Bromilow et al., 2016; Dupont et al., 2011; Labuschagne & Igrejas, 2020; Lexhaller et al., 2019; Vensel et al., 2002), proteome analytical tools allowing high-throughput analyses that can keep up with genotyping still need to be developed, especially for intact proteins. Therefore, the quantitative analysis of wheat proteins has so far been limited to comparatively small sample sets with 15–150 varieties (Afzal et al., 2021; Pronin, Börner, et al., 2020), in contrast to up to 80,000 accessions that were recently genotyped (Sansaloni et al., 2020). In the following, the focus is on studies that analyzed intact wheat gluten proteins, rather than peptides, as is the case in the most commonly used bottom-up proteomics experiments.

2.1 | Genetic factors

The genetic information underlying gluten proteins determines both their qualitative and quantitative composition. Common wheat, also known as bread wheat (*Triticum aestivum* L.), and spelt (*Triticum spelta* L.) are hexaploid wheat species (genome AABBDD). Durum wheat, well-known as pasta wheat (*Triticum durum* L.), and emmer (*Triticum dicoccum* [Schrank] Schübler) are tetraploid species (genome AABB), whereas einkorn (*Triticum monococcum* L.) is a diploid species (genome AA). Within each species, nucleotide deletions, insertions or mutations and different expression levels govern gluten protein composition and variation.

2.1.1 | Wheat species

To investigate the influence of species, a well-characterized set of eight varieties each of einkorn, emmer, durum wheat, spelt, and common wheat was used to characterize differences in the quantitative protein composition. All varieties were grown under standardized conditions at Seligenstadt, Germany, in the same year (Longin et al., 2016). The grains were dehulled in case of einkorn, emmer and spelt and milled to wholemeal flours before the determination of crude protein content ($N \times 5.7$) and content and proportion of albumins/globulins, gliadins and glutenins (Figure 1) (Geisslitz et al., 2018). The content (g/100 g of flour) of total proteins of common wheat (10.9, all values given as median in the following) was significantly lower than that of einkorn (12.8), spelt (12.9), and durum wheat (14.0). Further, durum wheat contained distinctly higher protein levels than common wheat and emmer (11.7).

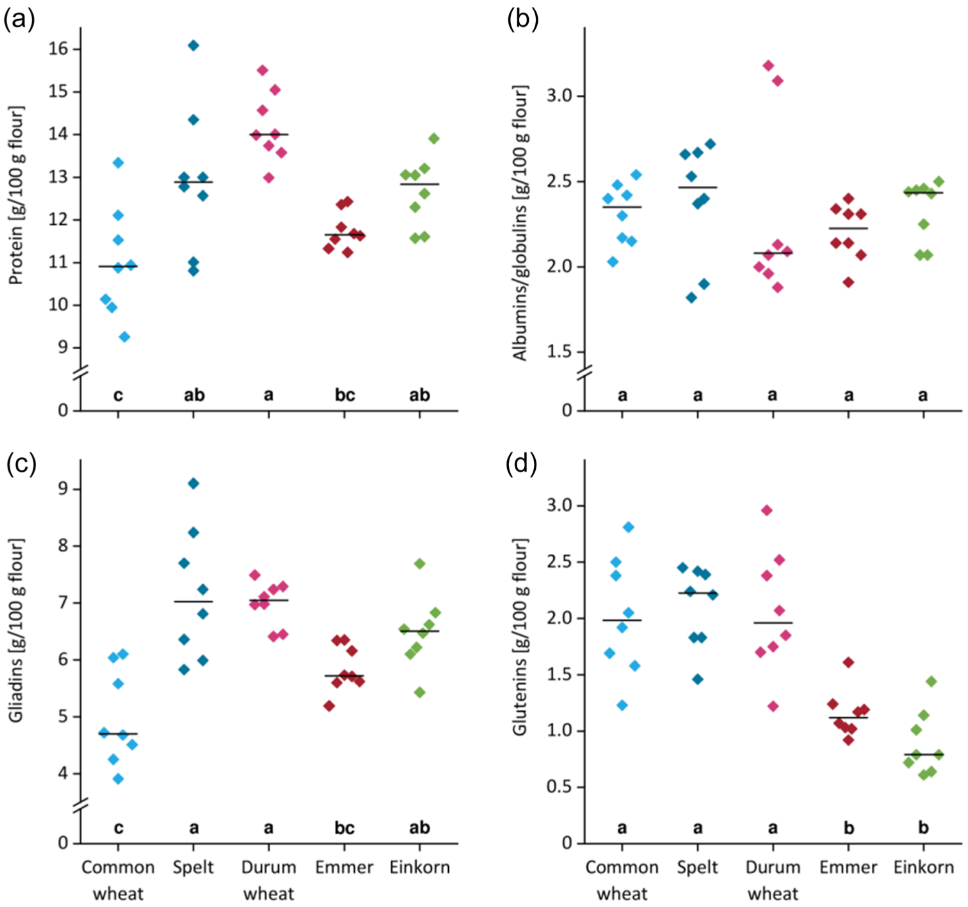

FIGURE 1 Content of crude protein (a), albumins/globulins (b), gliadins (c), and glutenins (d) in eight varieties each of common wheat, spelt, durum wheat, emmer, and einkorn, reported by Geisslitz et al. (2018). The line indicates the median and different small letters indicate significant differences between the wheat species (one-way analysis of variance [ANOVA], Tukey's test at $p < .05$) [Color figure can be viewed at wileyonlinelibrary.com]

The albumin/globulin content (2.1–2.5) was not significantly different. Regarding gliadins, the content increased from common wheat (4.7) to emmer (5.7), einkorn (6.5), durum wheat (7.0), and spelt (7.0). The lowest content of glutenins was present in einkorn (0.8) and emmer (1.1). In comparison, durum wheat (2.0), spelt (2.2), and common wheat (2.0) contained about twice the amount. The apparent differences between gliadin and glutenin content were well reflected by the gliadin/glutenin ratio that was lowest for common wheat (2.4) and highest for einkorn (8.1) with emmer (5.4), durum wheat (3.6), and spelt (3.4) in between. These findings were corroborated using a much larger sample set of 15 varieties per wheat species cultivated at four locations, that is, 300 samples in total. As expected based on the smaller sample set (Figure 1), the overall variability within each species increased further due to the additional environmental effect, but the trends described above were still present (Geisslitz et al., 2019).

The proportions of Osborne fractions relative to their sum showed a clear picture with gliadins as dominant fraction. Einkorn (67%) presented the highest proportion followed by durum wheat (65%), emmer (64%), spelt (61%), and common wheat (52%) with the lowest proportion. Glutenin values also indicated differences between species: einkorn (8%) and emmer (12%) had the lowest levels, durum wheat and spelt had intermediate levels (both 18%), and common wheat (21%) had the highest level. The proportions of albumins/globulins (19%–25%) were comparable and exceeded those of glutenins (9%–21%), due to the use of wholemeal flour. In white flour, the proportions of albumins/globulins are usually lower than those of glutenins (Wieser & Seilmeier, 1998).

Figure 2 shows the influence of species on single gluten protein types relative to total gluten proteins. In general, α-gliadins (33%–51%) were the predominant type, followed by γ-gliadins (24%–36%) and LMW-GS (10%–20%). ω5-Gliadins (3%–12%) and ω1,2-gliadins (2%–7%) belonged to the minor types while ωb-gliadins occurred in traces (0.4%–1.6%). Einkorn was most divergent from the other species, because the flours

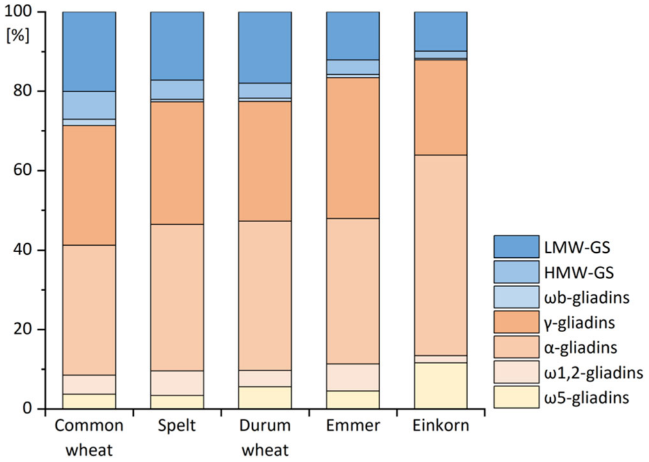

FIGURE 2 Proportions of gluten protein types relative to total gluten in whole grain flour of five wheat species. The values are means of eight varieties each, as reported by Geisslitz et al. (2018). HMW-GS, high-molecular-weight glutenin subunits; LMW-GS, low-molecular-weight glutenin subunits [Color figure can be viewed at wileyonlinelibrary.com]

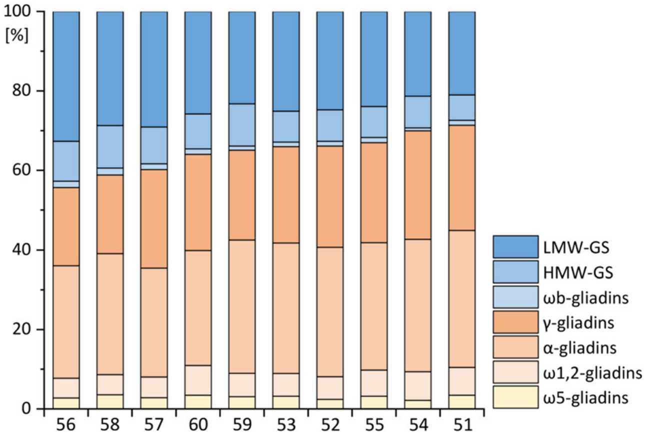

FIGURE 3 Proportions of gluten protein types relative to total gluten in whole grain flour of 10 German common wheat varieties (harvested in 2017, in Gatersleben, Germany), sorted by increasing gliadin/glutenin ratios, as reported by Pronin, Börner, et al. (2020). The numbers designate the different varieties: 51, Ritmo, 52, Zentos, 53, Astron, 54, Borenos, 55, Orestis, 56, Akteur, 57, Cubus, 58, Dekan, 59, Drifter, 60, Tommi. HMW-GS, high-molecular-weight glutenin subunits; LMW-GS, low-molecular-weight glutenin subunits. [Color figure can be viewed at wileyonlinelibrary.com]

contained significantly more α-gliadins (51%) and ω5-gliadins (12%), and less ω1,2-gliadins (2%), ωb-gliadins (0.4%), and γ-gliadins (24%) compared to the other species. Common wheat was characterized by the highest proportions of HMW-GS (7%) and ωb-gliadins (1.6%) and the lowest proportion of α-gliadins (33%). Emmer, durum wheat, and spelt showed similar compositions except the lower LMW-GS value (12%) and the higher γ-gliadin value (36%) of emmer. Genome-related differences were best reflected in the percentages of HMW-GS and ω5-gliadins.

Investigations on a different collection of 33 common wheat, nine spelt, six durum wheat, four emmer and seven einkorn samples revealed a higher overall protein content in most cases, with means ranging from 14.6 g/100 g_{DM} (based on dry matter) for durum wheat to 18.2 g/100 g_{DM} for einkorn (Call et al., 2020). The gluten composition reported by Call et al. (2020) for the five wheat species partially differed to that found by Geisslitz et al. (2018) as illustrated, for example, by gliadin/glutenin ratios of 1.1–1.3 for common wheat, spelt, durum wheat and einkorn, compared to 2.6 for emmer. With largely comparable fractionation and analytical procedures, this variation was attributed to the different genetic backgrounds of the samples and the environmental and crop management procedures (Call et al., 2020).

2.1.2 | Wheat variety

Within each species, wheat varieties display a high variability in their quantitative protein composition, even when grown under comparable conditions (Geisslitz et al., 2018) As an example, Figure 3 shows the genetic influence of the common wheat variety on single gluten protein types relative to total gluten proteins (Pronin, Börner, et al., 2020) The percentages of gliadins were 56%–71% and those of glutenins 29%–44%, resulting in gliadin/glutenin ratios of 1.3–2.5. Regarding types, the most noticeable differences were observed for HMW-GS (6%–11%), LMW-GS (21%–33%) as well as ω5-gliadins (2.1%–3.6%), ω1,2-gliadins (5.0%–7.5%), and ωb-gliadins (0.8%–1.7%). Compared to the traditional approach of integrating only gluten protein types together as a group, a new integration method designed to capture each peak improved the differentiation of variety-specific fingerprints and helped identify varieties with exceptional protein composition (Pronin, Geisslitz, et al., 2020).

2.1.3 | HMW-GS

Although HMW-GS usually make up less than 10% of gluten proteins, they play a key role in the formation of the gluten network and the baking quality of common wheat (Payne et al., 1987). HMW-GS are encoded at the *Glu-1* loci of group 1 chromosomes (1A, 1B and 1D) and each locus consists of two genes encoding an x-type and a y-type subunit. Common wheat varieties usually contain three, four, or five subunits, because some genes are silenced (mostly 1Ax and always 1Ay). The most frequent combinations of subunits are [1 + 5 + 7 + 9 + 10], [2* + 5 + 7 + 9 + 10], [5 + 7 + 9 + 10], [1 + 2 + 7 + 8 + 12], [2 + 7 + 9 + 12], [2 + 7 + 8 + 12], [2 + 6 + 8 + 12], [2* + 5 + 7 + 10], and [1 + 2 + 6 + 8 + 12] (Wieser & Zimmermann, 2000). Single subunits and allelic pairs have been ranked in order of baking quality with the allelic pair 1Dx5 + 1Dy10 as best combination, whereas 1Bx6 + 1By8 scored worst (Payne et al., 1987). For example, varieties with the combination [5 + 7 + 9 + 10] usually have better baking quality than varieties with [2 + 6 + 8 + 12]. In particular, the presence of subunits 1Dx5 and 1Bx7 is important due to their special disulfide structure (1Dx5 vs. 1Dx2) and the high amount (1Bx7 vs. 1Bx6) (Wieser et al., under review).

Many studies focus on the qualitative identification of HMW-GS in different wheat varieties and species and correlations to dough and bread quality traits (Geisslitz et al., 2020; Jang et al., 2017; Zhao et al., 2020), but only few actually report quantities of HMW-GS (Dupont et al., 2007; Wieser & Zimmermann, 2000). Dupont et al. (2007) determined HMW-GS molar fractions for three wheat varieties with the [2* + 5 + 7 + 9 + 10] combination and found 25%–32% of 1Bx7, 20%–27% of 1Dy10, 19%–20% of 1Dx5, 13%–18% of 1By9 and 13%–14% of Ax2*. Two varieties with the [2 + 7 + 8 + 12] combination contained an average of 37% of 1Bx7, 23% of 1Dx2, 21% of 1Dy12, and 18% of 1By8.

The proportions of single subunits related to total HMW-GS reported by Wieser and Zimmermann (2000) were highly variable (10%–41%) with lower values for subunits 1, 2*, 6, 8, and 9 compared to subunits 2, 5, 7, 10, and 12. Figure 4 illustrates the composition of nine varieties selected from the 28 analyzed in total. x-Type subunits (68%) were highly dominant in relation to y-type subunits (32%). The average results for all 28 international varieties investigated showed pronounced variety-specific variations, but some general observations were made: subunits 1Dx2 and 1Bx7 (both mean 35%) were major components, followed by subunits 1Dx5 (28%), 1Dy12 (21%), 1Dy10 (20%), and 1Bx6 (18%). Subunits 1Ax1 and 1By8 (both mean 14%), 1Ax2* (13%), and 1Ay9 (11%) belonged to the minor components.

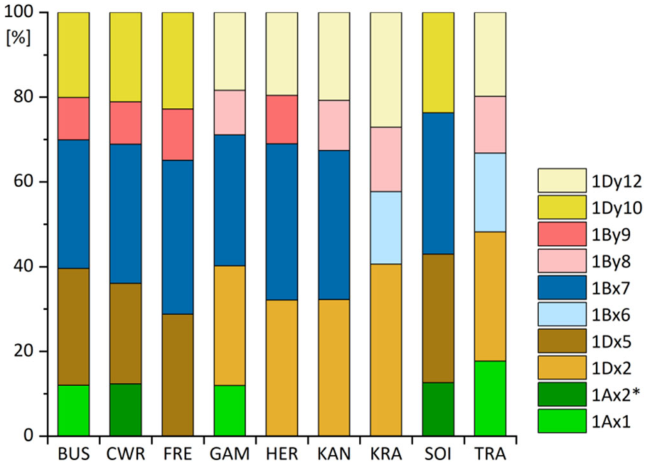

FIGURE 4 Proportions of single high-molecular-weight glutenin subunits (HMW-GS) related to total HMW-GS in nine different common wheat varieties including BUS, Bussard, CWR, Canadian Western Red Spring, FRE, Fresco, GAM, Gambrinus, HER, Hereward, KAN, Kanzler, KRA, Kraka, SOI, Soissons, and TRA, Transit, as reported by Wieser and Zimmermann (2000) [Color figure can be viewed at wileyonlinelibrary.com]

2.1.4 | Wheat breeding

In an effort to clarify the question whether wheat breeding might have contributed to the increasing prevalence of wheat-related disorders (WRD), a number of research groups have studied the effect of breeding on changes in gluten protein composition of common wheat (Call et al., 2020; Kasarda, 2013; Malalgoda et al., 2018; Ozuna & Barro, 2018; Pronin, Börner, et al., 2020; Ribeiro et al., 2016). Depending on the genetic background of the samples and the conditions of cultivation, different changes in gliadin, glutenin and/or gluten content were observed, but all studies essentially concluded that modern common wheat varieties were similar to old ones in terms of their potential to trigger WRD. Old varieties usually had a similar or even higher content of protein and gluten compared to modern ones (Kasarda, 2013; Malalgoda et al., 2018; Pronin, Börner, et al., 2020). Investigations of 60 German common wheat cultivars, first registered from 1891 to 2010 grown under the same conditions in three consecutive harvest years (2015, 2016, and 2017) showed that there was essentially no change in albumins/globulins and total gluten proteins. In contrast, glutenins increased whereas gliadins and gliadin/glutenin ratios decreased over the past 100 years (Figure 5) (Pronin, Börner, et al., 2020). Similar results with decreasing gliadin/glutenin ratios were also reported by Ozuna and Barro (2018). Regarding gluten protein types, there was no change of ω5-gliadins nor ω1,2-gliadins, but α- and γ-gliadins decreased, while both HMW-GS and LMW-GS increased. The increase in HMW-GS is certainly related to breeding efforts aimed at improving dough handling properties and bread-making performance.

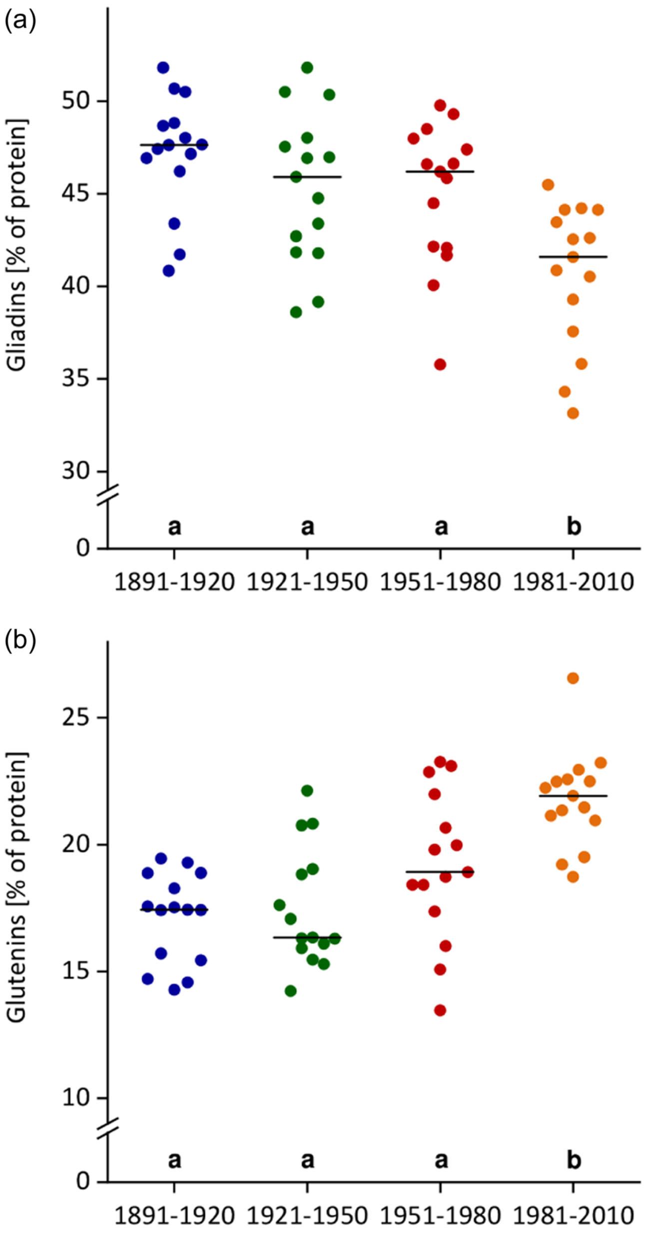

FIGURE 5 Proportions of gliadins (a) and glutenins (b) relative to total protein in whole grain flour of 60 German common wheat varieties first registered from 1891 to 2010 (harvested in 2015, 2016, and 2017, in Gatersleben, Germany), as reported by Pronin, Börner, et al. (2020). The line indicates the median and different small letters indicate significant differences between each group of 15 samples (one-way analysis of variance [ANOVA], Tukey's test at $p < .05$) [Color figure can be viewed at wileyonlinelibrary.com]

2.2 | Environmental factors

In addition to genetics, environmental factors contribute about two-thirds of variability regarding wheat yield and composition (Hellemans et al., 2018; Johansson et al., 2013). The stability of quality-related traits is desirable to manufacture end-products with constant and reproducible properties. Therefore, it is important to know how environmental factors affect the composition of wheat grains, especially regarding protein content and gluten composition.

2.2.1 | Soil type and tillage

Wheat is preferentially cultivated on loamy soil, but it can also grow in other soil types from sandy to heavy clay. One important characteristic is the water holding capacity of the soil and how well the wheat plant can extract water and nutrients. To study the effect of soil type on protein content and composition, three wheat varieties were grown on two different soil types, grey vertosol (medium-clay, regional good quality soil) and red kandosol (sandy clay-loam, less fertile than vertosol), using either complete or zero tillage, but equivalent nutrient concentration (Wilkes et al., 2010). Wheat grown on the grey vertosol had higher protein content (16%) compared to the red kandosol (11%). Complete tillage also led to higher protein content compared to zero tillage, but overall the effect of tillage on grain composition was limited, with the soil type having a much larger effect. The vertosol soil also caused an increase in insoluble proteins, among them gluten proteins, triticin and, most notably, β-amylase (Wilkes et al., 2010).

2.2.2 | Weather conditions

Advances in crop management techniques and varieties with improved resistance against abiotic stress factors have increased the stability of yield and quality-related traits, but wheat production in the field still remains highly susceptible to weather variations. Climate change with its associated extreme weather conditions ranging from drought to events of heavy rain puts crops under cold, heat, drought, water and/or frost stress, may influence their ability to absorb nutrients and will affect grain yield and composition (Pagani et al., 2017). For example, wheat yield predictions using the Agricultural Production Systems SIMulator with site-specific calibration were accurate across a wide range of environmental conditions, but overestimated yield when abiotic stress, such as drought or nitrogen (N) limitation occurred (Hao et al., 2021).

2.2.2.1 | *Temperature*

Temperature influences wheat protein composition with different effects depending on the growth stage. For example, heat induces a shorter grain-filling period with lower grain yield and relatively higher protein content (Dupont & Altenbach, 2003), with protein accumulation starting earlier and with faster rates (Dupont, Hurkman, Vensel, Tanaka, et al., 2006). Experiments with wheat variety Butte86 grown under three controlled day/night temperatures (24/17°C, 37/17°C, and 37/28°C regimens) showed that flour protein percentage increased 1.2-fold at 37/17°C and 1.9-fold at 37/28°C compared to 24/17°C. Regarding gluten protein composition, the percentage of gliadins remained unchanged, whereas that of glutenins increased slightly at 37/28°C compared to 24/17°C. Within glutenins, HMW-GS increased from about 13% to 15%, while LMW-GS were hardly affected, with only a slight decrease from 33% to 32%. Overall, the effect of different temperatures alone appeared to be small, compared to the changes induced by increasing levels of N fertilization that were studied in parallel (Dupont, Hurkman, Vensel, Chan, et al., 2006). A study on four wheat varieties grown in different seasons and at different sites in Norway found that higher mean temperature from heading to midway in the grain filling period was positively related to gluten quality, assessed by micro-scale extension tests. When the daytime temperature in this period fell below 18°C, gluten quality was inferior (Moldestad et al., 2011). Further experiments with two wheat varieties grown in climate chambers at 13/10°C and 23/20°C found significant temperature effects on gluten composition, with increasing proportions of ω-gliadins and D-type LMW-GS, decreasing proportions of α- and γ-gliadins and B-type LMW-GS with increasing temperature for both cultivars. The proportion of HMW-GS remained constant. However, the ratios of gliadin/glutenin, HMW-GS/LMW-GS and monomeric/polymeric proteins were not affected. The changes were variety-dependent with little effect on gluten quality seen for the Norwegian variety adapted to low temperatures, compared to the UK variety adapted to milder conditions (Koga et al., 2015). The percentage of sodium dodecyl sulfate (SDS)-unextractable polymeric proteins, the so-called glutenin macropolymer (GMP) was identified as important parameter related to gluten quality (Koga et al., 2016).

2.2.2.2 | *Drought*

When drought occurs together with heat, the duration of grain-filling is further reduced compared to either factor alone, with the largest effect seen with drought between days 1 and 14 after anthesis (i.e., flowering) (Gooding et al., 2003). Linear increases in percentages of gliadins and glutenins were observed with increasing flour protein content. However, gliadins increased more rapidly than glutenins, resulting in higher gliadin/glutenin ratios (Saint Pierre et al., 2008). In contrast, studies on six winter wheat lines and hybrids showed that the concentration of gliadins was slightly to severely (−20%) decreased with the occurrence of drought stress, whereas glutenins significantly increased in the lines but not in the hybrids. HMW-GS significantly increased in two hybrids and all lines while the sum of LMW-GS decreased slightly (Zörb et al., 2017). In-depth proteomics studies revealed that some gluten proteins including α-gliadins, γ-gliadins, and some LMW-GS decreased and other LMW-GS increased in abundance in response to drought. Several α-gliadins, γ-gliadins, and LMW-GS increased while other α-gliadins and ω-gliadins decreased in response to high temperature. The results indicated that stress could increase the percentage of some gluten proteins at the expense of other gluten proteins with different amino acid sequences. The underlying molecular mechanisms are still unknown, though (Yang et al., 2011).

2.2.2.3 | *Irrigation*

A field study in Turkey in two consecutive growing seasons compared the effects of a fully irrigated treatment, a rainfed treatment, an early, a late and a continuous drought treatment using one regional wheat variety adapted to drought. The fully irrigated treatment resulted in the highest grain yield (4459 kg/ha), but the lowest grain protein (10.5%) and wet gluten (28.8%) content. In contrast, continuous drought led to the lowest grain yield (1533 kg/ha) and the highest protein (12.4%) and wet gluten (35.1%) content. The other three treatments lay in between (Ozturk & Aydin, 2004). Another biennial study from China used three irrigation levels during the growing season: irrigation at jointing (I1, 75 mm of water), irrigation at before-wintering, jointing, and anthesis (I2, 225 mm of water), and irrigation at before-wintering, jointing, anthesis, and grain filling (I3, 300 mm of water). Grain yield was lower with treatment I1 (5925 kg/ha) compared to I2 (6862 kg/ha) and I3 (6651 kg/ha), but the effect of different irrigation levels on flour protein content was inconsistent between the two harvest years. Irrigation levels influenced glutenin biosynthesis and glutenin particle formation, with treatments I1 (drought) and I3 (excess water) resulting in less and smaller GMP, reduced HMW-GS content, reduced HMW-GS/LMW-GS ratio and smaller loaf volume compared to treatment I2 (Jia et al., 2012).

2.2.2.4 | *Light and shading*

Light intensity also has an impact on grain morphology, yield and starch and protein quality. A study with two strong Chinese winter wheat cultivars revealed that shading at different grain-filling stages all increased the content of each individual and total HMW-GS compared to no shading. GMP particles were larger when shading occurred at the middle (11–20 days after anthesis) and late (21–30 days after anthesis) grain-filling stages, whereas shading at early (1–10 days after anthesis) grain-filling stages induced smaller GMP particles, most likely due to differences in maturation periods (Chen et al., 2013). Furthermore, Li et al. (2012) reported that severe shading (77% of total radiation) reduced HMW-GS and GMP accumulation, but increased their content in the mature grain due to an overall decrease in grain weight compared to the control (full radiation). Vice versa, mild shading (92% of total radiation), increased HMW-GS and GMP accumulation, resulting in the highest total HMW-GS and GMP contents in the mature grain. The effects also depended on the cultivar.

2.2.3 | Atmospheric CO_2 concentration

The continuing increase in atmospheric CO_2 concentration from 417 ml/m^3 in 2021 to an estimated 550 ml/m^3 in 2050 is predicted to significantly decrease protein content for several crops, including wheat, barley, rice, and soybean (Taub et al., 2008). Elevated CO_2 (eCO_2) levels are associated with higher growth and yield, driven by increasing concentrations of C, but with lower concentrations of, for example, N, Fe, and Zn (Loladze, 2002). A recent meta-analysis found that eCO_2 levels resulted in a significant reduction in grain N concentration (−8.4%), but a significant increase in absolute amount of N (+12%), because of overall grain yield stimulation (Broberg et al., 2017). The effect was consistent over all experimental setups, only the magnitude was dependent on the setup, that is, free-air-CO_2-enrichment (FACE) showed a smaller effect compared to open-top chambers and field soil also resulted in smaller changes compared to pots. Quality-related traits, such as bread loaf volume, wet/dry gluten content, Zeleny sedimentation value and Hagberg falling number were all negatively affected (−5.8% to −21.2%) by eCO_2 (Broberg et al., 2017).

To study the effects of eCO_2 on wheat protein composition, common wheat variety Batis was grown under ambient air and FACE conditions (CO_2 concentration of 550 ml/m^3) and two different N fertilization levels with adequate N supply at 168 kg N/ha (N100) and reduced N supply at 84 kg N/ha (N50). Elevated CO_2 caused significant reductions in white flour protein content (N50: 6.7 vs. 6.1 g/100 g; N100: 9.1 vs. 7.8 g/100 g). The content of all protein fractions and types except albumins/globulins was significantly reduced under eCO_2 and the effect was more pronounced in the N100 samples compared to the N50 samples. The proportions of gluten protein types were not influenced by eCO_2 at the N50 level, whereas the proportions of ω5-gliadins (3.6% vs. 2.8%), ω1,2-gliadins (3.4% vs. 3.1%), α-gliadins (27.4% vs. 26.1%), and HMW-GS (9.5% vs. 9.0%) slightly decreased and those of γ-gliadins (30.5% vs. 31.1%) and LMW-GS (24.5% vs. 26.3%) increased under eCO_2 at the N100 level (Wieser et al., 2008). A 3-year FACE experiment essentially confirmed that eCO_2 significantly decreased wheat grain protein, total gluten protein and gliadin content, whereas the glutenin content was not significantly changed, but also lower (Högy et al., 2009). Further investigations on the interactions between eCO_2 and deficient (38 kg N/ha), adequate (190 kg N/ha), and excess (320 kg N/ha) N fertilization showed that higher N supply increased albumin/globulin, gliadin, and glutenin content with a more pronounced effect on gliadins and glutenins compared to albumins/globulins. In partial contrast to the other two studies, eCO_2 only caused slightly decreased albumin/globulin and total gluten content irrespective of N level, whereas loaf volume and gluten protein composition remained unaffected (Dier et al., 2020).

Studies on the individual and combined effects of eCO_2 and postanthesis heat stress (HT) on the grain quality of spring wheat confirmed that eCO_2 significantly reduced grain protein content compared to ambient CO_2, whereas HT caused the opposite effect compared to ambient temperature (AT). The combination of eCO_2 and HT thus resulted in higher grain protein content compared to the control at ambient CO_2 and AT, indicating that HT may have a more profound effect than CO_2 concentration. Regarding gluten composition, HT decreased the gliadin/glutenin ratio and increased the HMW-GS/LMW-GS ratio in relation to ambient CO_2 and AT, irrespective of the CO_2 concentration (Zhang et al., 2019).

A FACE study from Australia with six specific wheat varieties selected for enhanced N use efficiency, water use efficiency, root vigor and grain quality also found reduced grain and flour protein content (−12%), gliadin proportions (−4%), and loaf volume (−13%) with eCO_2 under rainfed conditions, while glutenin proportions remained constant and albumins/globulins increased (+9%). Therefore, further plant breeding efforts are required to increase water use efficiency and targeted N assimilation from the wheat stem and leaves into the grains to help mitigate the negative effects of eCO_2 on quality-related traits (Walker et al., 2019). Another concern is that eCO_2 has been shown to increase risk of *Fusarium* head blight (FHB) and associated higher deoxynivalenol (DON) contamination in wheat

(Blandino et al., 2020), because lower grain protein content is associated with higher susceptibility of wheat to *Fusarium* and N deficiency-induced biosynthesis of DON (Hay et al., 2020).

2.2.4 | Wheat diseases

Wheat yield and quality can be reduced by several diseases including infestation with parasites or infection with pathogens such as wheat rust, blotch diseases, and FHB. FHB, primarily caused by *Fusarium graminearum*, is one of the most prevalent wheat diseases worldwide, can cause up to 70% of yield reduction due to poor grain filling and may lead to additional losses in case the DON content exceeds the regulatory threshold of 1250 μg/kg in unprocessed wheat (Figueroa et al., 2018).

To investigate changes in the protein composition of the grains, three varieties of emmer (EM) and one common wheat (CW) variety Amaretto were artificially inoculated with a mixed *F. culmorum* and *F. graminearum* spore suspension during anthesis (EM1 and CW1) and compared with nontreated plants (EM0 and CW0) (Eggert et al., 2010). The resulting concentration of DON in grains was 2.9 mg/kg (EM1) and 3.7 mg/kg (CW1), respectively. The protein composition of both species was affected in a similar way (Figure 6). While the protein content of 14.1% for EM and 14.7% for CW was not influenced by the infection, the proportions of gliadins, glutenins and gluten protein types showed clear changes. The proportions of gliadins increased from 71% to 83% (EM) and from 67% to 81% (CW), respectively, while the proportions of glutenins decreased. These effects were less pronounced in ω5- and ωb-gliadins and most pronounced in LMW-GS. The gliadin/glutenin ratio was increased from 2.4 to 4.9 (EM) and 2.0 to 4.3 (CW), respectively, pointing to weakening of gluten. The partial degradation of gluten proteins, especially of glutenins, by *Fusarium* species was also observed by Bellesi et al. (2019), who reported variety-specific differences that could be related to the subunit composition. Serine-type proteases were identified in *Fusarium* species as causes for gluten degradation (Koga et al., 2019). In general, fungal infection has been shown to cause damage to gluten proteins, resulting in low baking quality.

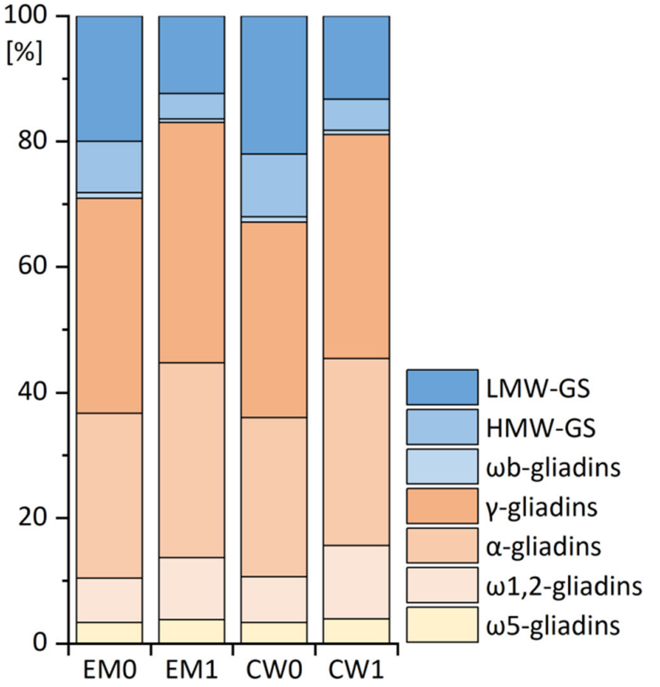

FIGURE 6 Proportions of gluten protein types relative to total gluten in whole grain flour of a mixture of three emmer varieties (EM) and one common wheat variety Amaretto (CW), both noninfected (0) and infected with *Fusarium* (1), as reported by Eggert et al. (2010). HMW-GS, high-molecular-weight glutenin subunits, LMW-GS; low-molecular-weight glutenin subunits [Color figure can be viewed at wileyonlinelibrary.com]

2.2.5 | Wheat fertilization

Fertilization is one of the most important determinants for wheat protein content and composition. Factors such as fertilization rate, splitting, number and timing of application and the form of fertilizer play a role and need to be adapted to wheat variety, soil type and meteorological conditions (Hellemans et al., 2018). Nitrogen (N) is an essential component of amino acids as building blocks of proteins and, therefore, N availability is an important requirement during all phases of wheat development to achieve high yield and quality. In general, increased N supply is associated with higher wheat productivity and grain protein content. However, excessive use of N fertilizer is also responsible for environmental issues such as nitrate leaching into groundwater, release of nitrous oxide and ammonia into the atmosphere and N accumulation in the soil. This is why N fertilizer application needs to be reduced by optimizing timing and rates of N fertilizers, by using and breeding wheat varieties with protein expression targeted for end-use quality and by focusing on yield stability (Zörb et al., 2018). Besides N, sulfur (S) is also essential for wheat growth, because it is needed for the synthesis of S-containing amino acids cysteine and methionine. In addition, wheat requires phosphorus, potassium and Cu, Mg, Zn, Fe, Mn, and so forth as micronutrients for plant growth, mitigation of biotic and abiotic stresses, and nutritional value of wheat flour. Excellent summaries of the different effects are already included in Hellemans et al. (2018) and Zörb et al. (2018), which is why the following will focus on studies how fertilization affects wheat gluten protein composition published between 2018 and 2022.

2.2.5.1 | *Total amount of N*

A study on 16 winter wheat varieties included field trials under two N fertilization levels (0 kg N/ha [N0] and 100 kg N/ha, applied as top dressings of 50 kg N/ha each at tillering and stem extension [N100]) in two years and three locations in Croatia. The results revealed statistically significant differences for the main factors (variety, N level, year, and location) and their interactions, with wheat variety being the main factor responsible for changes in quality-related proteins. Grain protein content increased from an average of 11.6% (N0) to 12.8% (N100), whereas albumins/globulins decreased by 1.1% overall at N100 compared to N0. Total gliadins, α-gliadins, γ-gliadins, and HMW-GS increased by 1.9%, 3.7%, 1.3%, and 0.9%, respectively, at N100, while total glutenins, LMW-GS, and ω-gliadins decreased by 1.8%, 3.0%, and 1.7%, respectively (Horvat et al., 2021). Consequently, the gliadin/glutenin ratio increased by 4.6% in response to high N supply, inter alia, because the effect of N level was mostly larger for the major protein types, such as α-gliadins and LMW-GS, compared to the minor types, such as HMW-GS, consistent with earlier reports (Wieser & Seilmeier, 1998).

The accumulation of gluten proteins in one Chinese winter wheat variety was studied at two N levels, N240 (240 kg N/ha) and N180 (180 kg N/ha, normal level). The proportions of total gliadins and glutenins including all types were higher at grain maturity with the N240 level compared to N180 (Zhen et al., 2020). Landolfi, D'Auria, et al. (2021) studied the effect of N fertilization rate (N80, 80 kg N/ha and N160, 160 kg N/ha, applied as top dressings split equally at tillering and stem elongation) on the gluten protein composition of one old and one modern wheat variety. Higher N supply resulted in an increase of grain protein content from 17.1% to 18.2% for the landrace and from 14.5% to 15.2% for the modern variety. As reported for the Chinese wheat variety (Zhen et al., 2020), the percentages of all gluten protein types also remained stable at both N levels, indicating that the individual components increased to a similar extent (Landolfi, D'Auria, et al., 2021).

Subsequent field experiments under rainfed conditions with three old wheat varieties from Italy analyzed the effect of seeding density (90 and 180 kg seed/ha), three N levels (35, 80, and 135 kg N/ha with 20% applied at seeding, 40% at tillering, and 40% at stem elongation) and two levels of foliar S fertilization (0 and 6.4 kg S/ha) on the protein composition and dough and bread properties. Seeding density had no significant effect on the parameters investigated. S fertilization decreased the percentage of albumins/globulins and gliadins, but increased the percentage of glutenins and total gluten proteins and improved dough workability compared to the treatment without S addition. Regarding N levels, only the highest N treatment (N135) resulted in significantly higher percentages of total protein, total gluten proteins, insoluble proteins and albumins, whereas globulins, gliadins and glutenins were not significantly affected compared to the lowest N level (N35). Seeding density and S fertilization did not significantly affect bread texture and volume, while higher N levels improved textural characteristics, indicating that optimized N fertilization could be useful to enhance flour processability (Guerrini et al., 2020).

To study the effect of N fertilization on gluten protein accumulation in two near-isogenic lines (NIL) with HMW-GS variation at the *Glu-D1* locus, four different N levels (0, 60, 90, and 120 kg N/ha) were applied at booting. The application of increasing N levels accelerated the accumulation of total protein, gliadins, glutenins, and GMP during grain-filling and resulted in higher contents at maturity. The gliadin/glutenin ratio decreased, while the HMW-GS/LMW-GS ratio increased with higher N supply. The effect was more pronounced in the *Glu-D1a* NIL with subunits Dx2 and Dy12 associated with a weaker gluten network, compared to the *Glu-D1d* NIL with subunits Dx5 and Dy10 associated with a strong gluten network and good baking quality. N application at booting was thus suitable to improve the structural and thermal properties of wheat gluten, especially in the NIL with inferior subunit composition (Song et al., 2020).

Two South-African hard red spring wheat cultivars were treated with low N and low phosphorus and a combination of both in greenhouse experiments to study the influence on the composition of monomeric and polymeric proteins as well as HMW-GS (Tóth et al., 2019, 2020). All treatments, but especially the low N treatment, significantly reduced the SDS-insoluble polymeric proteins, that is, total glutenins, HMW-GS and LMW-GS, whereas SDS-soluble proteins and SDS-insoluble small and large monomeric proteins, that is, gliadins, increased (Tóth et al., 2020). The percentages of HMW-GS 1Ax1, 1By7, and 1By8 encoded by *Glu-A1* and *Glu-B1* loci significantly increased under low N, while those of 1Dx2 remained stable or decreased in case of 1Dy12. The combination of low N and low phosphorus resulted in similar changes except that the increase in 1By7 was not significant compared to the control (Tóth et al., 2019). Overall, the low N treatment and the combination had a much larger effect on gluten composition than the low phosphorus treatment (Tóth et al., 2019, 2020).

The effects of water deficiency, N fertilization and their combination on gluten protein composition and bread-making quality of a Chinese wheat variety was studied in field experiments with 120 and 240 kg N/ha (normal and high-N) and with well-watered irrigation and water deficit. High-N and water deficit alone and combined led to significant increases in protein, total gluten protein, and GMP content compared to the normal-N and well-watered control. The content of total gliadins did not change relative to the control under both treatments alone, but the combination resulted in a significant increase, mainly due to an increase in α- and γ-gliadins. In contrast, the content of total glutenins increased with high-N, water deficit and the combination, as did HMW-GS, LMW-GS and the four individual subunits 1Bx7, 1By9, 1Dx2, and 1Dy12. Finally, these changes in gluten protein composition also affected the flour bread-making properties, as loaf volume was increased by 65% with high-N alone and by 62% with the combined treatment and thus more than with water deficit alone (J. Liu et al., 2022).

2.2.5.2 | *Timing of N application*

Apart from the total amount of N, timing of N application is known to be important, because the same amount of N split over different growth stages enhances N use efficiency compared to a single dose. Application at late growth stages also promotes protein formation in the grain (Bogard et al., 2010). To study changes in protein composition, two German winter wheat varieties were cultivated in pots with two different N treatments: early-N with 1 g N/pot applied before seeding and 1 g N/pot at the beginning of stem elongation compared to split-N with 1 g N/pot applied before seeding, 0.5 g N/pot at the beginning of stem elongation and 0.5 g N/pot at the late booting stage. Grain yield was not significantly affected by the N treatments, whereas grain protein content increased by 4%–9% and bread loaf volume by 5%–11% depending on the variety with split-N compared to early-N. Proteome analysis with two-dimensional gel electrophoresis revealed about 350 protein spots, of which 21 and 28 spots in each variety were significantly changed depending on the N treatment. These spots included globulins, LMW-GS, α- and γ-gliadins, α-amylase/trypsin inhibitors and other enzymes and these protein-specific changes were variety-dependent (Xue et al., 2019).

Another study investigated two bread-making and one feed wheat variety grown in field experiments with four N treatments: 100 kg N/ha (N100) and 200 kg N/ha (N200) without and with additional late foliar N application at anthesis (40 kg N/ha), respectively. Grain yield increased at N200 compared to N100, but foliar N did not cause further significant increases in most cases. The average grain protein content increased from 9% (N100) to 10% (N100 + foliar N) and from about 11% (N200) to 12% (N200 + foliar N). Regarding bread loaf volume, the most interesting effect was that the average volume at N100 + foliar N was comparable to N200. The gluten protein composition analyzed via SDS-PAGE indicated that foliar N application did not change the percentage of ω-gliadins, while HMW-GS increased and LMW-GS/α-/γ-gliadins decreased. Complementary size-exclusion (SE)-high-performance liquid chromatography (HPLC) analyses revealed that the gliadin/glutenin ratio stayed constant, whereas the HMW-GS/LMW-GS ratio decreased and the gliadin/HMW-GS ratio increased when comparing N100 + foliar N against N100. Taken together, the study showed that additional N fertilization resulting in higher overall grain protein content does not necessarily lead to higher loaf volume, but that an optimal treatment needs to be determined for each variety to maximize bread-making quality, also considering its gluten protein composition (Rossmann et al., 2019). The poor correlation between grain protein content and loaf volume as well as the importance of gluten protein composition were essentially confirmed in a follow-up study. Furthermore, baking quality was improved with low late N application (additional 60 kg N/ha at the late booting growth stage) compared to no additional late N (only 50 kg N/ha at seeding and 100 kg N/ha at the beginning of stem elongation), and a high late dose (additional 80 kg N/ha at the late booting growth stage) did not increase loaf volume further (Rossmann et al., 2020).

Investigations from Northern Italy over a period of three years found that late N fertilization (additional 30 kg N/ha) increased the grain protein content by 1.2% compared to the control (fertilized with 50 kg N/ha at tillering and 80 kg N/ha at the beginning of stem elongation). However, the gluten protein composition did not change significantly, because all gluten protein types were affected in the same way. The concomitant application of fungicide at heading caused a slight reduction in grain protein content (−0.3%), but again, gluten composition remained unaffected. In contrast, the environmental conditions during each of the three years had the largest effect on gluten protein composition (Landolfi, Visioli, et al., 2021).

The timing of N topdressing was studied in more detail using one Chinese winter wheat variety fertilized with 120 kg N/ha before sowing plus 120 kg N/ha either at the emergence of the top-fifth leaf (TL5), of the top-third leaf (TL3, at jointing) or of the flag leaf (TL1).

Wheat flour protein content significantly increased from 13.9% (TL5) to 14.4% (TL3) and to 15.8% (TL1). Gliadins and glutenins both increased in a comparable way, so that gliadin/glutenin ratios showed no significant alteration (0.89–0.93) (Zhong et al., 2018). Proteomics using the isobaric tag for relative and absolute quantitation (iTRAQ) technique identified 591 proteins from 17 different functional classes in wheat flour, of which 50 and 63 were differentially expressed in TL5 and TL1 compared to TL3. Of the 50 differently expressed proteins in TL5 compared to TL3, 20 proteins related to DNA binding, redox reactions, metal binding, and adenosine triphosphate (ATP) binding were upregulated, while 30 proteins associated with storage (especially one ω-gliadin, six γ-gliadins, and four HMW-GS), amino acid metabolism and pathogen defense were downregulated. Of the 63 differently expressed proteins in TL1 compared to TL3, 38 proteins related to protein synthesis, assembly or processing and storage (one ω-gliadin, three γ-gliadins, three HMW-GS, and one LMW-GS) were upregulated, whereas 25 proteins associated with ATP binding, C metabolism and redox reactions were downregulated. In total, earlier N topdressing (TL5) negatively affected gluten protein composition compared to the recommended timing (TL3) and late N topdressing (TL1) showed positive effects on gluten protein composition (Zhong et al., 2019).

2.2.5.3 | *N application strategy*

In addition to the total amount of N and timing of N application, the N application strategy was studied for its impact on wheat quality-related parameters. The impact of supplying the total amount of N by repeated foliar application was compared to conventional soil fertilization using one Italian wheat variety with high breadmaking quality grown in two seasons (Ferrari et al., 2021). All treatments received 32 kg N/ha at sowing and there was one control with no further fertilization. The conventional treatment (C-M) involved two top dressings of 58 kg N/ha at tillering and stem elongation, respectively, plus 12 kg N/ha as foliar application at anthesis (160 kg N/ha in total). Three different foliar treatments with 120, 104, and 96 kg N/ha in total were carried out with 8, 16, 32, and 32 kg N/ha (F-120), 8, 16, 32, and 16 kg N/ha (F-104), and 16, 16, 16, and 16 kg N/ha (F-96) applied at tillering, stem elongation, booting, and anthesis, respectively, all as urea. Grain protein content was quite similar in year 1 with 13.2% (F-96) to 14.7% (F-104). In year 2, it was between 12.0% (F-96) and 14.2% (F-120). No statistically significant difference was observed for the content of HMW-GS in both years, whereas LMW-GS either decreased by up to 19% or increased by up to 35% compared to C-M. Gliadins increased significantly by 12% to 13% in both years following the F-120 treatment compared to C-M. Urea was also compared at the same doses with urea-ammonium-nitrate in one season and the form of N also had different effects on grain protein content and gluten composition. The study showed that foliar N application with urea was preferable compared to urea-ammonium-nitrate. Quality-related traits were similar in the wheats with conventional and foliar treatments, indicating that foliar N application allows a reduction of total N addition by 25%–40% (Ferrari et al., 2021).

De Santis et al. (2020) studied the effect of seven N fertilization strategies on gluten protein content and composition as well as other quality-related parameters of two red soft wheat cultivars for biscuit production grown in three consecutive seasons. All treatments amounted to 130 kg N/ha in total, but varied in timing of application, in presence or absence of S and in type of N fertilizer, that is, urea for slower release compared to ammonium and rapid release from nitrate (Table 1). Grain protein content ranged from 8.8% to 11.0% overall and was significantly influenced by N fertilization. Taking the data from all three seasons together, AN-AS resulted in the highest grain protein content (10.8%), followed by AN-AN (10.3%) and AN-U (10.0%) (for abbreviations, please refer to Table 1). The slow release treatments resulted in slightly lower grain protein content (9.5%–9.8%) compared to AN-AN as standard. The growing season and the N fertilization strategy both affected the gliadin/glutenin ratio, with the lowest average of 0.94 for the AN-AS treatment and the highest value of 1.67 for the SR-OM strategy. Grain protein content and the gliadin/glutenin ratio showed a significant negative correlation ($r = -0.61$). The two varieties also responded differently to the seven treatments and this is why well-designed studies such as this one are essential to assess the respective effects of growing season, variety and treatment as well as the interactions between these factors (De Santis et al., 2020).

2.2.5.4 | *Sulfur*

Sulfur is important for wheat growth, grain protein synthesis and regulation of N metabolism. Soil sulfur deficiency is common in many wheat-growing regions due to a reduction of sulfur emissions from industrial production and intensive agricultural systems that lead to sulfur depletion (Blake-Kalff et al., 2000). The study by De Santis et al. (2020) already showed that a combined application of N and S (AN-AS) increased grain protein content, but decreased gliadin/glutenin ratios compared to N application alone (AN-AN) (Table 1). More detailed investigations into the impact of soil sulfur deficiency on the wheat protein composition of four Australian

TABLE 1 Nitrogen fertilization strategies, grain protein content and gliadin/glutenin ratio presented as means of two wheat varieties studied

Treatment	N source		N [kg/ha]		S [kg/ha]		GPC [%]			Gliadin/glutenin ratio		
	GS 23	GS 32	GS 23	GS 32	GS 23	GS 32	Y1	Y2	Y3	Y1	Y2	Y3
AN-AN	Ammonium nitrate	Ammonium nitrate	50	80	0	0	9.8	10.7	10.3	1.32	0.96	0.93
AN-AS	Ammonium nitrate	Ammonium sulfate	50	80	0	65	11.0	10.9	10.6	0.85	0.99	0.97
AN-U	Ammonium nitrate	Urea	50	80	0	0	9.4	10.4	10.2	1.29	1.02	1.19
SR-NI	Ammonium nitrate + nitrification inhibitor	-	130	0	0	0	9.3	10.1	9.8	1.39	1.11	0.95
SR-NSI	Ammonium sulfate nitrate + nitrification inhibitor	-	130	0	65	0	9.3	10.0	10.0	1.66	1.51	1.13
SR-DC	Slow release fertilizer, double membrane	-	130	0	22	0	8.9	9.6	10.0	1.29	1.44	1.23
SR-OM	Slow release fertilizer, organic-mineral	-	130	0	26	0	8.8	9.6	10.1	2.18	1.78	1.06

Note: Data rearranged from De Santis et al. (2020).

Abbreviations: GPC, grain protein content, GS 23, application at tillering; GS 32, application at stem elongation; Y1, growth season 2013/14; Y2, growth season 2014/15; Y3, growth season 2015/16.

varieties showed that supplementation treatments with 30 kg S/ha (S30) and 50 kg S/ha (S50) increased protein yield by up to 33% and N use efficiency by up to 35% compared to the control without sulfur (S0) (Yu et al., 2021). Total gluten content did not change significantly, but the percentage of gliadins decreased by up to 22% with S30 compared to S0, but to a smaller percentage when S50 was compared to S0 (Figure 7). In comparison to S0, the percentages of ω5-gliadins decreased by up to 38% at S30 and by up to 84% at S50. The percentages of ω1,2-, α-, and γ-gliadins were lowest at the S30 level with decreases of up to 26% compared to S0, but increased again at the S50 level to reach percentages comparable to S0 in case of ω1,2- and α-gliadins. Vice versa, glutenin percentages increased by up to 87%, which is also reflected in the glutenin to gliadin ratios. Sulfur supplementation increased the activity of glutamine synthetase 1 in developing grains, especially from 28 to 35 days postanthesis, indicating that more free glutamine was available for gluten protein synthesis during grain development (Yu et al., 2021).

2.2.5.5 | *Phosphorus, potassium, and micronutrients*

Next to N, phosphorus is the second most important mineral nutrient used by plants. An optimal combination of N and phosphorus fertilization is essential to optimize plant growth and maximize yield (Hu & Chu, 2020). Studies on the effect of phosphorus on wheat gluten composition are quite rare, maybe because the studies so far found that phosphorus only had a minor influence on wheat grain composition (Hellemans et al., 2018). Both reports by Tóth et al. essentially confirmed that the low phosphorus treatment had much smaller effects on all protein-related parameters compared to the low N treatment (Tóth et al., 2019, 2020). Some changes were observed in the content of SDS-soluble proteins that increased and in the proportion of insoluble large monomeric proteins, that is, mainly gliadins, that decreased in response to low phosphorus levels (Tóth et al., 2020).

Potassium (K) is essential for wheat development and K deficiency appears to be as limiting as N and phosphorus for plant productivity, especially in dry and semi-dry regions (Sardans & Peñuelas, 2015). To study the effect of foliar K application on wheat protein content and composition, two Chinese wheat varieties were grown in field trials in two seasons with 150 kg/ha urea and 100 kg/ha diammonium phosphate as basal fertilizers (Gu et al., 2021). Foliar application was performed by spraying 30 mmol/L KCl (K1), 60 mmol/L KCl (K2), and 90 mmol/L KCl (K3) at booting, anthesis and 10 days after anthesis, respectively, with water spraying as

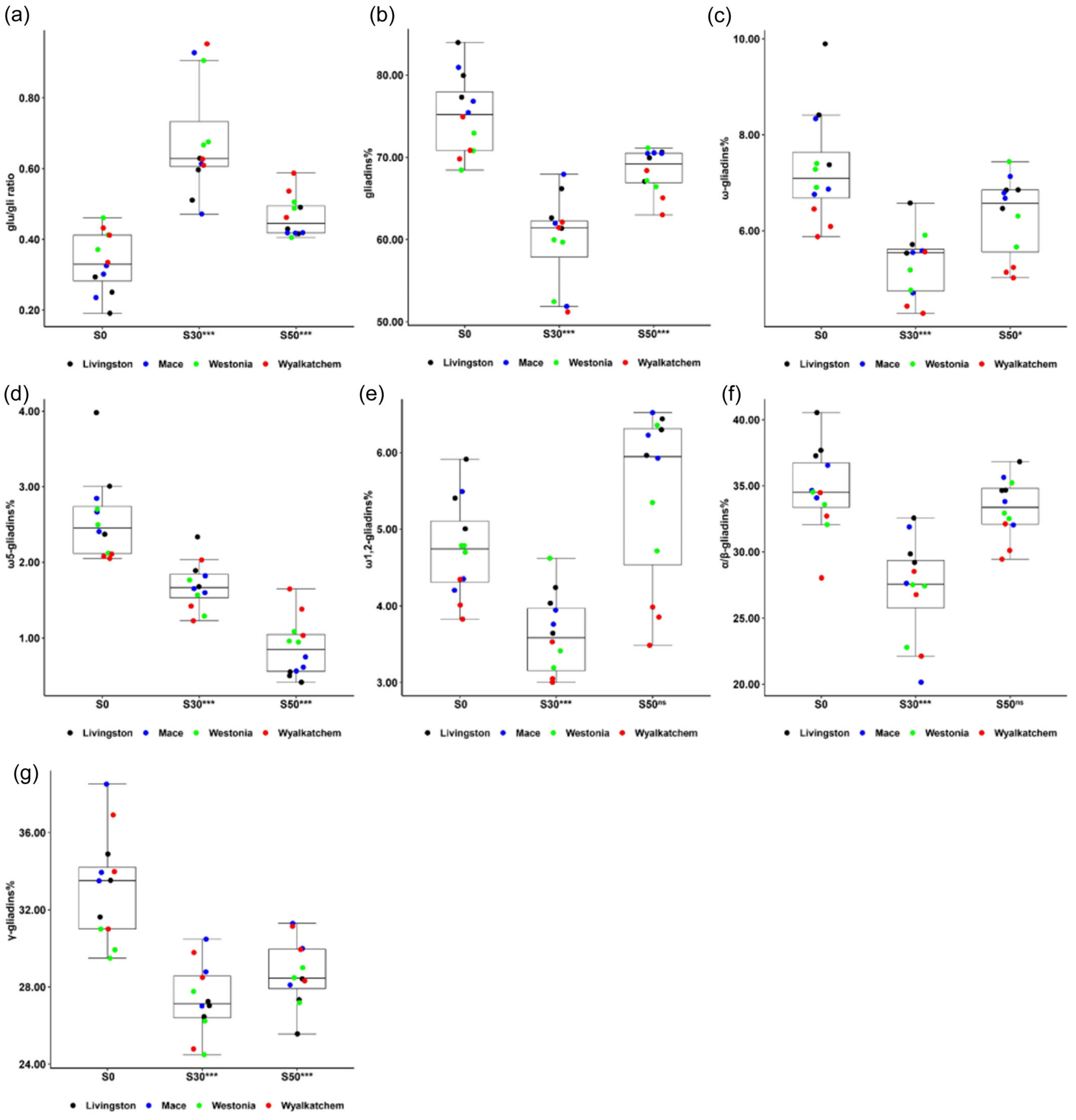

FIGURE 7 Influence of sulfur treatment in the field trial on the ratio of glutenins to gliadins (a), the percentage of gliadins (b) and the percentage of ω-gliadins (c), ω5-gliadins (d), ω1,2-gliadins (e), α-gliadins (f) and γ-gliadins (g) relative to total gluten content. S0, S30, and S50 represent 0, 30, and 50 kg/ha sulfur treatments, respectively; **p* < .05; ***p* < .01; ****p* < .001; ns, not significant. Three biologically independent replicates (or plot) for each cultivar under each sulfur treatment were analyzed. The boxplot shows the median and interquartile ranges. Black, blue, green, and red dots show the data distribution of the four cultivars Livingston, Mace, Westonia, and Wyalkatchem under each sulfur treatment. The figure is taken unmodified from Yu et al. (2021), available under a Creative Commons Attribution 4.0 International License: https://creativecommons.org/licenses/by/4.0/ [Color figure can be viewed at wileyonlinelibrary.com]

control. The K2 treatment at anthesis resulted in the highest grain protein content for both varieties with increases of 8% and 7% compared to the control, respectively. Yield was mostly unaffected. In terms of protein composition, foliar K application in comparison to the control slightly reduced albumins, but did not significantly affect globulins. Both gliadins and glutenins increased by up to 16% and 17%, respectively, with the largest effect seen with the K2 treatment either at anthesis or 10 days postanthesis, depending on each variety. Individual HMW-GS were either unaffected by foliar K application, or significantly increased, with the largest effect (up to +24%) seen in case of Ax1 in response to K2 treatment at anthesis. Especially the application of 60 mmol/L KCl at anthesis also improved dough rheological properties, so that foliar K application was considered to be beneficial for wheat quality (Gu et al., 2021).

Micronutrients such as Cu, Mg, Zn, Fe, Mn, and others are important for the nutritional value of wheat for human consumption. Fertilizers containing micronutrients and new breeding strategies may be used to increase the uptake of minerals from the soil during plant growth and achieve biofortification of wheat flours and products (Borrill et al., 2014; Wang et al., 2020). Fe and Zn deficiency are still widespread and affect up to two billion people worldwide. Wheat and other cereal products contribute up to 44% and 25% of the daily intake of Fe and Zn, respectively (Shewry, 2009), which is why adequate concentrations of these minerals are essential to combat malnutrition. Next to the positive effect on human nutrition, Zn fertilization with 5–20 mg Zn/kg soil has been shown to increase grain and flour protein content of three Chinese wheat varieties by increasing albumins, globulins, gliadins, and glutenins. The results were variety-dependent and indicated that 10–20 mg Zn/kg soil were the optimal dose, because a higher dose (40 mg Zn/kg soil) led to a subsequent decrease of all protein components (H. E. Liu et al., 2015). Tao et al. (2018) investigated the effect of four levels of Zn fertilizer (0, 15, 30, and 45 mg Zn/kg soil) combined with normal temperature or high-temperature stress (HTS, 38°C for 2 days) at 20 days postanthesis on wheat protein content and composition. Total protein, albumin, gliadin, and glutenin content increased with HTS compared to normal temperature, whereas grain yield and globulin content decreased. The addition of Zn fertilizer increased grain yield, total protein, albumin, gliadin, and glutenin content, but decreased globulin content. In agreement with H. E. Liu et al. (2015) the largest effect on grain yield and protein composition was observed at an addition level of 15 mg Zn/kg soil. The negative effects of HTS on wheat quality were partially mitigated by Zn fertilization, but the underlying regulatory mechanisms still need to be clarified (Tao et al., 2018).

2.2.5.6 | *Biofertilizers*

The use of plant-aiding microorganisms such as plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungi, so-called biofertilizers, is one promising possibility to enhance sustainability and reduce the use of conventional fertilizers and pesticides during plant cultivation. Through their interaction with plant roots, they increase, for example, plant productivity, mineral uptake, drought tolerance, and disease resistance (Zvinavashe et al., 2021), but there is little knowledge of the effects of biofertilizers on wheat grain quality so far. This is why Dal Cortivo et al. (2020) inoculated wheat seeds with a bacterial consortium (*Azospirillum* spp., *Azoarcus* spp., and *Azorhizobium* spp.; called TN) and two mycorrhizal fungal-bacterial consortia, that is, R-N (*Rhizophagus irregularis* and *Azotobacter vinelandii*) and R-PK (*R. irregularis*, *Bacillus megaterium*, and *Frateuria aurantia*) and compared these with noninoculated controls. All biofertilizers significantly increased plant growth and N accumulation, but there were no significant changes in grain yield. Flour gluten content also remained stable independent of the treatment, with only small variations in gluten protein composition. The largest effect was caused by inoculation with R-PK, that led to an increase in gliadins and LMW-GS by 7% each and a reduction in HMW-GS by 29%, resulting in an overall decrease of glutenins by 8%. SDS-PAGE showed that one specific LMW-GS ($M_r \approx 43{,}600$) and one Bx HMW-GS were upregulated, most likely due to higher N supply. This may translate to an increase in gluten quality, but further investigations are needed, because no rheological or baking properties were assessed (Dal Cortivo et al., 2020). One recent study that compared three different N levels in conventional and organic cultivation of hard red winter wheat found that biofertilizers had little to no effect on baking quality or yield. However, the organic samples had lower baking volume when compared to conventional samples, so that further work is required to use potential synergies between manure-based fertilizer and biofertilizers, especially for organic farming (Al-Zubade et al., 2021).

3 | FUTURE PERSPECTIVES

Protein content is the benchmark indicator of wheat quality for national and international trade and subsequent processing. Depending on the final product, different flour specifications are required, such as 7%–10% of protein for cakes or pastries compared to 12%–14% of protein for bread. Due to the consistent negative relationship between yield gain and protein content, protein accumulation needs to be enhanced through fertilization, especially with N. However, environmental concerns limit the application of fertilizers, so that a variety of strategies are needed to maximize protein yield while minimizing excess N that leaches unused into the soil, the water and the atmosphere.

Recent evidence suggests that a reduction of up to 40% of N-based fertilizers is possible without loss of wheat quality by adapting application management and optimizing times, rates and types of fertilizer (Zörb et al., 2018). In doing so, the focus should not be on N alone, but rather the combination of N, S, phosphorus, potassium, and other micronutrients adapted to the specific needs of each variety in each particular environment.

Wheat genetics can help identify gluten protein genes that do not contribute to quality-related traits but consume N resources. Such genes could be silenced or removed using targeted genome editing techniques, such as CRISPR-Cas9. Further, varieties with high yield stability and improved resistance against abiotic and biotic stresses will be more suitable to ensure consistent protein content and quality (Zörb et al., 2018).

The large number of studies on quality-related changes in wheat gluten composition depending on various factors included in this review suggests that there are many groups working in this field, but only few of them in a collaborative way. First, only few wheat varieties are typically studied from the huge diversity of wheat varieties grown worldwide.

Second, comparisons between different studies are almost impossible, because of the huge variability in experimental setups, environmental conditions and varieties studied. Setups may or may not include appropriate controls, and may include one or several treatments, often with too few repetitions to account for confounding factors, for example, due to different climatic conditions from sowing to harvest. This is one of the reasons why different studies often report inconsistent results, for example, that the gliadin/glutenin ratio increased with a higher total amount of N (Horvat et al., 2021; Wieser & Seilmeier, 1998) or did not change (Landolfi, D'Auria, et al., 2021; Zhen et al., 2020). The more factors are varied, the harder it becomes to clarify the underlying metabolic pathways in the wheat plant because different stress factors will lead to various compensation mechanisms with upregulation or downregulation of certain proteins including enzymes, enzyme inhibitors or gluten proteins.

Third, the parameters that are investigated often vary between the studies. Yield and grain protein content are reported most widely, sometimes accompanied by other agronomic characteristics (thousand kernel weight, harvest index, etc.). Often different methods (RP-HPLC, GP-HPLC, SDS-PAGE, etc.) are used to study gluten protein composition and there are no harmonized protocols that allow a proper comparison of extraction, measurement and data evaluation procedures. Some studies also include dough rheological and baking parameters, but again with different instruments, such as farinograph, extensograph, alveograph, gluten index, and so forth.

To help address these issues, the Wheat Initiative was founded to coordinate strategic activities for wheat research at the international level. Expert Working Groups (EWG) within the Wheat Initiative have been established for seed proteins, carbohydrates & nutrients, allergy, food safety, and genetic resources & gene nomenclature. One of the achievements of the EWG so far has been to select and distribute representative master sets of varieties to use when studying certain traits, for example, LMW-GS. Furthermore, a repository was compiled for standardized methods such as SDS-PAGE, acid-PAGE, and DNA markers. The Wheat Initiative also helps to coordinate international collaborations and improve the link between genetics and genomics research and germplasm resources (Ikeda, 2020). With ongoing progress in gluten protein characterization, this link can be further extended to proteomics and parameters related to processing and baking quality. The development of common guidelines on how to set up experiments, for example, to study the effect of one specific factor on gluten composition would also be helpful to improve comparability and reproducibility of the results. This review may therefore be useful as an up-to-date compilation of the progress in wheat gluten-related research so far and spark new ideas for further research to improve wheat quality for processing, nutrition and health.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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