Research Article

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Grain quality traits within the wheat (*Triticum* spp.) genepool: prospects for improved nutrition through *de novo* domestication

Frederike Zeibig,[a] Benjamin Kilian,[b] Hakan Özkan,[c] Sumitra Pantha[a] and Michael Frei[a*]

Abstract

BACKGROUND: Wild relatives of wheat (*Triticum* spp.) harbor beneficial alleles for potential improvement and *de novo* domestication of selected genotypes with advantageous traits. We analyzed the nutrient composition in wild diploid and tetraploid wheats and their domesticated diploid, tetraploid and hexaploid relatives under field conditions in Germany and compared them with modern *Triticum aestivum* and *Triticum durum* cultivars. Grain iron (Fe) and zinc (Zn) concentrations, phytate:mineral molar ratios, grain protein content (GPC) and antioxidant activity were analyzed across 125 genotypes.

RESULTS: Grain Fe and Zn concentrations in wild wheats were 72 mg kg^{-1} and 59 mg kg^{-1}, respectively, with improved bioavailability indicated by Phytate:Fe and Phytate:Zn molar ratios (11.7 and 16.9, respectively) and GPC (231 g kg^{-1}). By comparison, grain Fe and Zn concentrations in landrace taxa were 54 mg kg^{-1} and 55 mg kg^{-1}, respectively, with lower Phytate:Fe and Phytate:Zn molar ratios (15.1 and 17.5, respectively) and GPC (178 g kg^{-1}). Average grain Fe accumulation in *Triticum araraticum* was 73 mg kg^{-1}, reaching 116 mg kg^{-1}, with high Fe bioavailability (Phyt:Fe: 11.7; minimum: 7.2). Wild wheats, landraces and modern cultivars showed no differences in antioxidant activity. *Triticum zhukovskyi* stood out with high grain micronutrient concentrations and favorable molar ratios. It was also the only taxon with elevated antioxidant activity.

CONCLUSION: Our results indicate alteration of grain quality during domestication. *T. araraticum* has promising genotypes with advantageous grain quality characteristics that could be selected for *de novo* domestication. Favorable nutritional traits in the GGAA wheat lineage (*T. araraticum* and *T. zhukovskyi*) hold promise for improving grain quality traits.

Supporting information may be found in the online version of this article.

Keywords: crop wild relatives; micronutrient bioavailability; grain quality; *Triticum araraticum*; *Triticum zhukovskyi*; wheat

INTRODUCTION

Wheat (*Triticum* spp.) is cultivated at almost all latitudes[1,2] and provides approximately 18% of total dietary calories and 19% of the protein requirements of the global population.[1,3] As the second most widely consumed cereal after rice, wheat is an essential source of micronutrients and other bioactive compounds.[1,2,4] Its nutritional contribution is particularly important in countries where food diversity is low and wheat is the primary crop consumed. Hidden hunger may result if it does may not provide sufficient micronutrients. Iron (Fe) and zinc (Zn) deficiencies are widespread, leading to impaired cognitive skills, physical activity and development.[4,5] Therefore, biofortification of wheat grains is a major target that can be achieved by introducing agronomic practices and breeding new cultivars.[6] Foods with high levels of antioxidant activity, entailing scavenging of radical oxygen species, have positive health effects, preventing chronic diseases. Among them, wholewheat foods are important sources of bioactive compounds that confer antioxidant activity. In bread wheat, phenolic compounds constitute the majority of antioxidants, of which ferulic acid is the most abundant phenolic acid.[7] Given that wheat provides these diverse nutrients, its grain quality is of considerable importance.

Hexaploid bread wheat (*Triticum aestivum* L., $2n = 6x = 42$, BBAADD) emerged through allopolyploidization and is related to diploids and tetraploids. Diploid taxa include *Triticum urartu*

* *Correspondence to: M Frei, Department of Agronomy and Crop Physiology, Institute of Agronomy and Plant Breeding I, Justus-Liebig-University, 35392 Giessen, Germany. E-mail: michael.frei@agrar.uni-giessen.de*

a *Department of Agronomy and Crop Physiology, Institute of Agronomy and Plant Breeding I, Justus-Liebig-University, Giessen, Germany*

b *Global Crop Diversity Trust, Bonn, Germany*

c *Department of Field Crops, Faculty of Agriculture, University of Çukurova, Adana, Turkey*

Thumanjan ex Gandilyan ($2n = 2x = 14$, A^uA^u), the A genome donor of *T. aestivum*, and *Triticum boeoticum* Boiss. ($2n = 2x = 14$, A^bA^b, wild Einkorn wheat), which was domesticated as *Triticum monococcum* L. ($2n = 2x = 14$, A^bA^b, domesticated Einkorn wheat). Tetraploid taxa include *Triticum dicoccoides* (Körn. ex Asch. et Graebn.) Schweinf ($2n = 4x = 28$, BBA^uA^u, wild Emmer wheat), a wild tetraploid taxon that was domesticated as *Triticum dicoccon* Schrank ($2n = 4x = 28$, BBA^uA^u, domesticated Emmer wheat) and *Triticum durum* Desf. ($2n = 4x = 28$, BBA^uA^u). A second wild tetraploid taxa is *Triticum araraticum* Jakubz. ($2n = 4x = 28$, GGA^tA^t), which belongs to the GGAA wheat lineage. *Triticum araraticum* is considered to have been domesticated as *Triticum timopheevii* (Zhuk.) Zhuk ($2x = 4n = 28$, GGA^tA^t), although its domestication history may be more complex.[8] *Triticum timopheevii* was further hybridized with *T. monococcum* to form the hexaploid *Triticum zhukovskyi* Menabde et Ericzjan ($2n = 6x = 42$, $GGA^tA^tA^bA^b$).[9-11] The Emmer wheats are more closely related to *T. aestivum*, whereas the GGAA wheats belong to the secondary genepool of *T. aestivum*.[12,13] Thus, the *Triticum* genepool evidently harbors wide diversity.

This genetic diversity may offer prospects for improving grain quality. In previous studies, Einkorn and Emmer wheats showed greater variation in grain Fe and Zn concentrations compared to modern wheat cultivars.[6] None of the analyzed domesticates reached the grain Zn concentration found in wild Emmer.[5,14] Hence, the grain Zn concentration has apparently decreased with the transformation from wild to domesticated Emmer wheat, with wild Emmer wheat evidencing significant potential for Zn biofortification. Apart from *T. dicoccoides*, other wild wheats of the *Triticum* genepool could be potential sources for improving grain quality in wheat.[9] Similar to grain micronutrient concentrations, grain protein content (GPC) is higher in wild and domesticated landrace wheats than in modern wheats.[15-18] A reduction in GPC may have occurred during domestication, which increased yield and grain size.[19] GPC is positively associated with the gluten content in wheat[17] and is thus an important parameter of end use. Furthermore, *T. dicoccon* and *T. monococcum* have been proposed as potential crops for use in low-input agriculture with less fertilizer, given their relatively high protein yield efficiency.[17] Antioxidant activity in wheat relatives has previously been analyzed in wild Einkorn[20] and wild Emmer.[21,22] Wild Einkorn wheats showed higher total phenolic content (TPC), with wild Emmer wheats showing lower phenolic content than tetraploid domesticated taxa.[22] To the best of our knowledge, no study has compared diverse wild *Triticum* taxa with their domesticated landraces through analyses of samples all grown in the same environment, with the aim of singling out the effect of domestication on grain quality and excluding environmental influences. Furthermore, although the GGAA wheats and *T. urartu* have received little attention, they could harbor beneficial grain quality characteristics.

The process of introgressing favorable traits from wild plants into modern cultivars is time-consuming and entails several cycles of backcrossing to eliminate unfavorable wild traits.[23-25] *De novo* domestication, which is the modification of domestication alleles in wild species via genome editing, alters the wild species itself into a cultivated plant.[26,27] By overcoming breeding barriers, it potentially simplifies the usage of wild germplasm and the secondary genepool in domestication. Furthermore, it reduces unintended loss of genes during the lengthy domestication period.[28] However, selected genotypes for *de novo* domestication should have substantial advantages over established cultivars.[29] Other prerequisites are suitability for the prospective environment, the identification of suitable domestication gene orthologs, and the availability of annotated reference genomes and transformation protocols.[26,30,31] Therefore, phenotyping for advantageous traits and selecting favorable genotypes are crucial processes for developing appropriate genome editing approaches.

In the present study, we analyzed the grain quality of a diverse set of wild and domesticated wheat taxa grown in the same field under central European climatic conditions. Consistent with our aim of identifying suitable candidate genotypes for *de novo* domestication, we developed the following hypotheses. (i) Grain quality is altered by domestication, which is observable as differences in quality between wild wheats and their domesticated landrace taxa. (ii) GGAA wheats harbor untapped potential in terms of grain quality traits for wheat improvement. We selected suitable genotypes according to their grain quality characteristics for a *de novo* domestication project. The results of this project will help to elucidate the scope of using wild wheats to develop diversified and balanced diets, offering an alternative pathway for wheat breeding.

MATERIALS AND METHODS

Plant material and cultivation

The wheat genepool panel used in the present study comprised four wild taxa with 89 genotypes, six landrace taxa with 30 genotypes and six modern cultivars, covering a total of 125 genotypes (Table 1; see also Supporting information, Table S1). The wheats were classified as suggested in a previous study.[11] The modern cultivars used for the study were four winter wheat cultivars: cv. Apostel (I.G. Pflanzenzucht), cv. Julius (KWS SAAT SE & Co.), cv. Nordkap (SAATEN UNION) and cv. RGT Reform (RAGT Saaten), with two modern *T. durum* cultivars: cv. Sambadur (Hauptsaaten) and cv. Wintergold (SAATEN UNION).

During the 2020/2021 season, plants were grown in pairs in two blocks of a field (7 × 36 m, soil type: Fluvic Gleyic Cambisol, 40 kg Nmin ha^{-1}) at the Weilburger Grenze research station in Giessen, Germany (50°36′N, 8°39′E). The long-term average annual precipitation and average annual air temperature during 1981–2010 were 700 mm $year^{-1}$ and 9.7 °C, respectively. During the experiment, total precipitation from the time of planting in November 2020 until harvesting in August 2021 was 613.7 mm, and the mean temperature was 9.4 °C. Four single seeds per genotype were pre-cultivated in a greenhouse for 2 weeks in October before transferring them to the field. Two seedlings per genotype were planted in each block. No fertilizer was applied during cultivation. All plants were covered with a large Crispac microperforated plastic bag (Baumann Saatzuchtbedarf, Waldenburg, Germany) at heading date to catch shattering seeds and assign them to single plants. Plants were harvested individually during July and August. Specific descriptions of the cultivation method have been provided previously.[32] For each genotype, two plants, one from each block, were used as biological replicates.

Preparation of samples

Hulled grain samples were threshed as previously described.[32] Whole-grain samples were ground for 2 min at a frequency of 30 Hz at a mill (Mill MM400; RETSCH, Haan, Germany). Wolfram carbide containers were used to prevent iron contamination. Whole-grain samples were divided into two samples: one for digestion, phytate and GPC analysis, and a second for extraction of phenolics. The latter was stored at 4 °C prior to extraction. All

Table 1. Taxa, number of genotypes, biological status and ploidy level of materials used in the present study

Taxon name	No. genotypes	Biological status	Ploidy level	Genome
Wild taxa				
Triticum boeoticum	6	Wild	2*x*	A^b
Triticum urartu	5	Wild	2*x*	A^u
Triticum dicoccoides	8	Wild	4*x*	BA
Triticum araraticum	70	Wild	4*x*	GA^t
TOTAL	**89**			
Landrace taxa				
Triticum monococcum	7	Landrace	2*x*	A^b
Triticum dicoccon	12	Landrace	4*x*	BA
Triticum timopheevii	3	Landrace	4*x*	GA^t
Triticum durum	2	Landrace	4*x*	BA
Triticum aestivum	4	Landrace	6*x*	BAD
Triticum zhukovskyi	2	Landrace	6*x*	GA^tA^b
TOTAL	**30**			
Modern wheats				
Triticum durum cv.	2	Modern cultivar	4*x*	BA
Triticum aestivum cv.	4	Modern cultivar	6*x*	BAD
TOTAL	**6**			

biological replicates of each genotype were replicated twice reserved for further analysis.

Digestion of samples

Finely ground samples were oven-dried at 80 °C for 4 h prior to analysis and stored in a desiccator. For the digestion of samples, 300 mg of the whole-wheat ground sample were weighed in tubes. A green tea standard with a known concentration of elements (IVA Analysetechnik, Meerbusch, Germany) and two blanks were included in each round of digestion. Three milliliters of Millipore water (Millipore, Burlingotn, MA, USA) and 3 mL of 69% HNO_3 were added. Samples were digested at 180 °C for 30 min (20 min of heating to reach 180 °C and 10 min at 180 °C) in a microwave (Multiwave 5000; Anton Paar) and then cooled down to 70 °C. The cooled digested samples were then diluted to 50 mL using Millipore water and filtered (MN 280 ¼ filter paper; Macherey-Nagel GmbH, Düren, Germany). The filtered samples were stored at 4 °C before being analyzed their Fe, Zn and phosphorus (P) concentrations.

Measurement of iron and zinc concentrations

Fe and Zn concentrations in the filtered samples were measured with an atomic absorption spectrometer (AAS, SpektrAA 220FS; Varian, Mulgrave, Australia). The wavelengths used were 248.3 nm for Fe and 213.9 nm for Zn. Each sample was measured in duplicate.

Phytate extraction and analysis

Phytate was extracted and measured using a microplate reader-adapted protocol.[33] Briefly, 50 mg of powdered whole wheat were weighed in Eppendorf tubes with two replicates, and 1 mL of 3.5% HCl was added. The samples were vortexed and then sonicated for 1 h. They were vortexed again and centrifuged for 10 min at 15 000 × *g* to obtain the supernatant, which was stored at 4 °C prior to analysis. Wade reagent ($FeCl_3$ and sulfosalicylic acid) was used to measure phytate. Twenty microliters of extract, 280 µL of distilled water and 100 µL of wade reagent were mixed and vortexed. Next, 100 µL of the solution were transferred to a 96-well microplate with three replicates, and the absorbance was measured at 500 nm in an Infinite M Plex microplate reader (Tecan Group Ltd., Männedorf, Switzerland). To determine the bioavailability of micronutrients, we calculated the phytate: micronutrient molar ratio using the molecular weights of phytate (660.04 g mol^{-1}), Fe (55.845 g mol^{-1}) and Zn (65.38 g mol^{-1}).

Phosphorus measurement

A spectrophotometric method[34] adapted to the microplate format was used to measure P content. The color reagent was prepared as follows. For the stock reagent, 70.5 mL of concentrated H_2SO_4 was diluted in 500 mL of distilled water. Next, 6 g of ammonium heptamolybdate tetrahydrate and 0.14 g of potassium antimony(III) oxide tartrate trihydrate were each dissolved in 125 mL of Millipore water. The three mixtures were combined and made up to 1 L. For the color reagent, 100 mL of the stock solution were combined with 0.53 g of pure ascorbic acid. Next, 10 µL of the sample were added to a 96-well microplate, followed by 200 µL of sterile H_2O and 40 µL of color reagent, and incubated for 15 min. The extinction was measured at 578 nm in a microplate reader. All samples were analyzed in triplicate.

Grain protein content

To measure GPC, the ground samples were dried at 105 °C for 4 h and samples of 4–5 mg were weighed into tin boats. Each sample was measured in duplicate. The samples were burned at 1100 °C using an elemental analyzer (UNICUBE trace, Elementar Analysensysteme GmbH, Langenselbold, Germany) to determine nitrogen content, which was multiplied by 5.7, following ISO/TS 16634–2:2009, to determine GPC.

Extraction of phenolics

Ground samples were dried at 30 °C for 5 h and stored in a desiccator. Phenolics were extracted as previously described.[35] In brief,

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200 mg of samples (in duplicate) was weighed and 4 mL of acidified methanol (HCl/methanol/water, 1:80:10 v/v/v) was added, followed by placement in an ultrasound bath for 2 h. To ensure a temperature below 40 °C, ice was added every 20–30 min. Afterwards, the samples were centrifuged at 5000 × *g* for 5 min. Three milliliters of the supernatant were collected and stored at −20 °C prior to analysis.

Measurement of TPC

To measure the TPC, we followed an established protocol.[36] One hundred microliters of extracted sample, blank or standard gallic acid solution were mixed with 200 µL of 10% Folin–Ciocalteu reagent and vortexed. After 5 min, 800 µL of 700 mM of sodium carbonate were added, and the solution was incubated for 2 h after further vortexing. Afterwards, 200 µL of the sample were transferred to a 96-well microplate (Greiner Bio-One, Kremsmünster, Austria) and the absorbance was read at 765 nm. Each sample was measured in triplicate, and the results were calculated as gallic acid equivalents.

Oxygen radical absorbance capacity

Oxygen radical absorbance capacity (ORAC) was measured using the previously derived extracts and following an established protocol.[37] The extracts were diluted 1:50 prior to analysis. In brief, 150 µL of 0.08 µM fluorescein were added to a black 96-well microplate (Fluotrac; Greiner Bio-One). Twenty-five microliters of 75 mM phosphate buffer, Trolox standard solution or diluted sample were added for four replicates of the sample. The phosphate buffer served as blank. The microplate was preheated at 37 °C for 10 min and 25 µL of 150 mM 2,2′-azobis-(2-amidinopropane) dihydrochloride was added to each well. The fluorescence was read immediately for 1 h at excitation and emission wavelengths of 485 nm and 530 nm, respectively. The temperature in the microplate reader was maintained at 37 °C. The results were obtained in Trolox equivalents.

Statistical analysis

We conducted analysis of variance (ANOVA), with taxa or biological status considered as fixed effects and the block as a random effect (Table S2). For each taxon, the mean of all genotypes was calculated for the respective parameter. Pearson's correlation coefficients and significant associations were calculated. For the correlation and principal component analysis (PCA), we added the grain weight per plant and thousand kernel weight (TKW) reported in our previous study.[32] Statistics were performed in R Studio with R, version 4.2.0 (www.r-project.org). The emmeans (https://cran.r-project.org/web/packages/emmeans/index.html), multcomp (https://cran.r-project.org/web/packages/multcomp/index.html) and nlme (https://cran.r-project.org/web/packages/nlme/index.html) packages were used for the ANOVA, and the FactoMineR (https://cran.r-project.org/web/packages/FactoMineR/index.html), factoextra (https://cran.r-project.org/web/packages/factoextra/index.html) and corrplot (https://cran.r-project.org/web/packages/corrplot/index.html) packages were used for the PCA. Correlations were analyzed with the psych (https://cran.r-project.org/web/packages/psych/index.html) and corrplot packages. All data for each plant were used for correlation analyses and visualized with ggplot2 (https://ggplot2.tidyverse.org) and Inkscape (https://inkscape.org).

RESULTS

Iron and zinc grain concentrations and estimated bioavailability

From wild wheats to landraces, to modern cultivars, the grain concentrations of both Fe and Zn decreased (Fig. 1A). Grain Fe and Zn concentrations in the wild wheats significantly exceeded those of the modern cultivars. Between the wild wheat and landrace taxa, the difference was only significant for Fe concentrations (Fig. 1B). The landrace taxa and modern cultivars did not differ significantly for either Fe or Zn concentrations (Fig. 1B,D). However, the grain of landrace taxa showed high diversity for Zn concentrations (Fig. 1A). The wild wheats showed high diversity for both micronutrients (Fig. 1A). Their grain Fe and Zn concentrations ranged between 44 mg kg^{-1} and 116 mg kg^{-1} and between 27 mg kg^{-1} and 85 mg kg^{-1}, respectively.

The grain Fe concentration in the wild taxa was highest for the diploid taxa *T. boeoticum* (mean: 79 mg kg^{-1}) and *T. urartu* (mean: 76 mg kg^{-1}), followed by the tetraploid *T. araraticum* (mean: 73 mg kg^{-1}), and lowest in *T. dicoccoides* (mean: 61 mg kg^{-1}). Of all the taxa, the tetraploid, *T. durum* cv., had the lowest grain Fe concentration. A reducing trend for grain Fe concentrations from wild to domesticated offspring was observed for the following pairs: *T. boeoticum* to *T. monococcum*, *T. araraticum* to *T. timopheevii*, and *T. dicoccoides* to *T. dicoccon* (Fig. 1B).

The wild taxa showed significantly higher Fe bioavailability compared with the landraces and modern cultivars (Fig. 1C). Species of wild taxa did not differ significantly from each other, but the diploid species *T. boeoticum* and *T. urartu* showed the highest Fe bioavailability, followed by the tetraploids, *T. araraticum* and *T. dicoccoides*. The GGAA wheats, *T. araraticum*, *T. timopheevii* and *T. zhukovskyi* showed similarly high Fe bioavailability, with *T. araraticum* ranking among the taxa with the highest grain Fe concentration. However, compared with the high variation in grain Fe concentration in *T. araraticum*, bioavailability showed less variation (Fig. 1C). *Triticum durum*, *T. durum* cv. and *T. aestivum* showed low bioavailability for the grain Fe concentration.

The wild taxa showed significantly higher grain Zn concentrations than the modern varieties (Fig. 1D). Variations in grain Zn concentrations were high in both the wild and landrace taxa (coefficients of variation were 22.5% and 36.1% for wild and landrace taxa, respectively). In the wild taxa, this variation was mainly associated with *T. dicoccoides* and *T. araraticum*. Among the landrace taxa, *T. monococcum* and *T. dicoccon* showed some outliers with higher grain Zn concentration, but overall, *T. zhukovskyi* showed the highest grain Zn concentration. In general, grain Zn concentration was evenly distributed among the different taxa. *T. araraticum* and *T. boeoticum* tended to have elevated grain Zn concentrations compared with their derived domesticated taxa (*T. timopheevii* and *T. monococcum*, respectively).

For Zn bioavailability, *T. zhukovskyi*, which had the highest grain Zn concentration, also had the highest bioavailability (Fig. 1E). *T. durum* cv., *T. aestivum* cv. and *T. aestivum* (landrace) showed a high Phytate:Zn molar ratio, despite having the lowest grain Zn concentration.

GPC

GPC decreased significantly from wild taxa to landraces to modern cultivars. GPC was above 200 g kg^{-1} in all the wild taxa (Fig. 2), of which *T. araraticum* had the highest value, significantly exceeding that of *T. dicoccoides*. GPC did not vary widely in wild

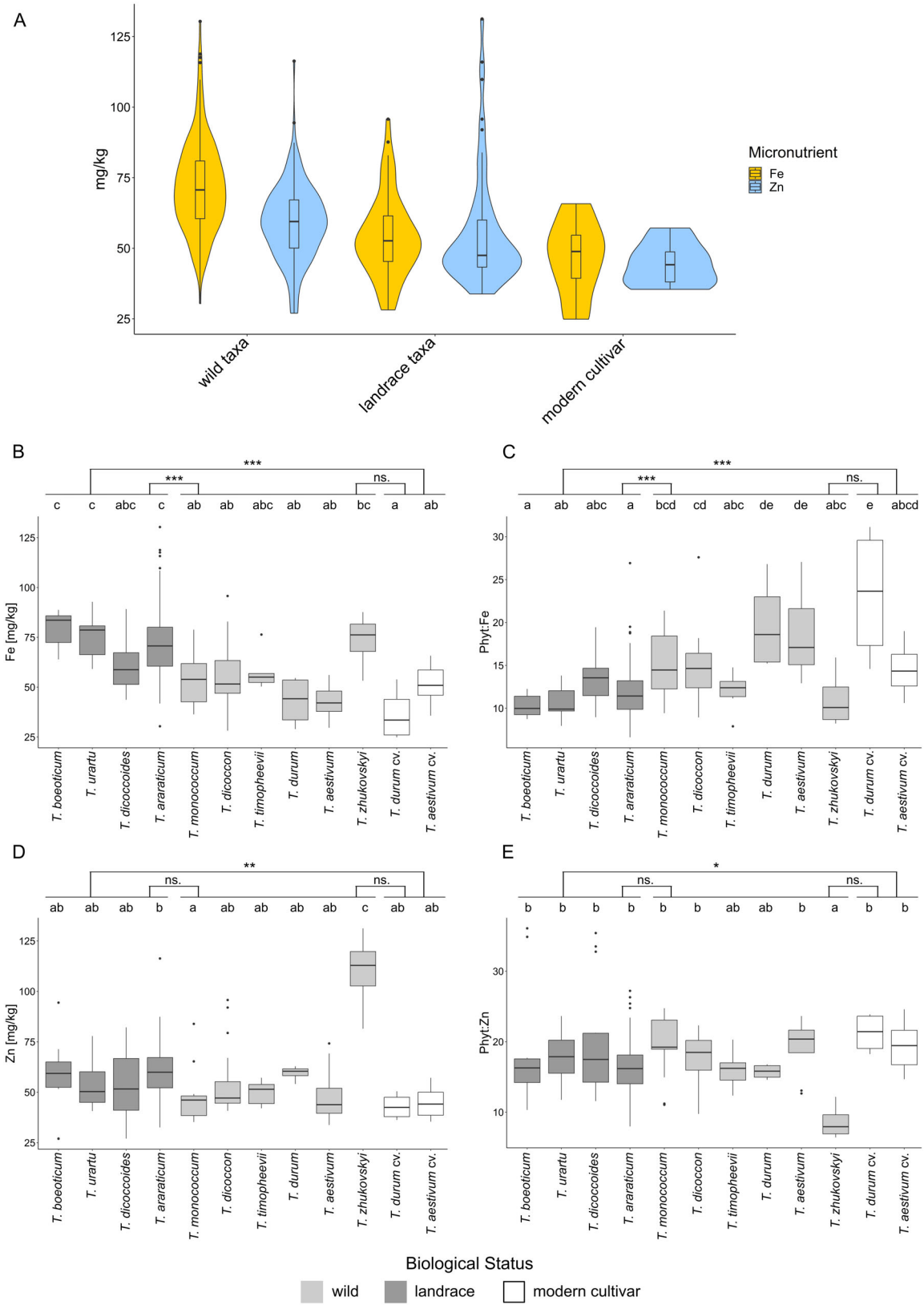

Figure 1. Fe and Zn grain concentrations and their bioavailability in wild taxa, landraces and modern cultivars. (A) Distribution of micronutrient concentrations according to biological status (wild, landrace or modern cultivar). (B) Fe grain concentrations (mg kg^{-1}) in wild taxa, landrace taxa and modern cultivars. (C) bioavailability of Fe indicated by Phyt:Fe ratios in wild taxa, landrace taxa and modern cultivars. (D) grain Zn concentration (mg kg^{-1}) in wild taxa, landrace taxa and modern cultivars; (E) Zn bioavailability indicated by Phyt:Zn ratios in wild taxa, landrace taxa and modern cultivars. Asterisks indicate significant differences between groups according to their biological status (*$P < 0.05$: **$P < 0.01$; ***$P < 0.001$); ns. no significant difference. Different letters indicate significant differences among taxa ($P < 0.05$).

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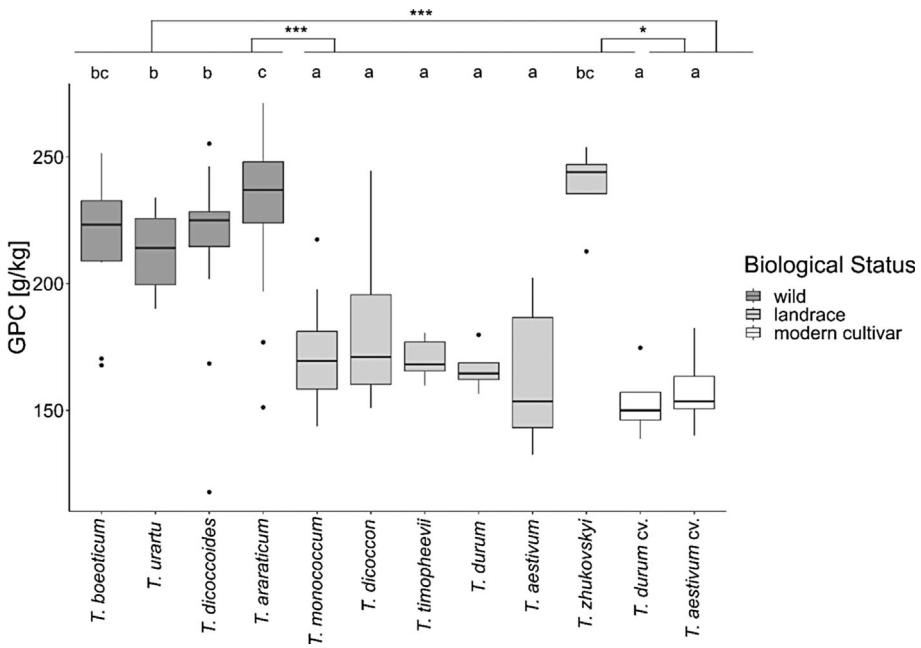

Figure 2. Grain protein content (GPC) of wild taxa, landraces and modern cultivars. Asterisks indicate significant differences between groups according to their biological status (*$P < 0.05$: **$P < 0.01$; ***$P < 0.001$); ns, no significant difference; Different letters indicate significant differences among taxa ($P < 0.05$).

taxa (coefficient of variation: 9.8%) and was significantly higher than that in most landraces. *T. zhukovskyi* was exceptional, having the highest GPC values, which differed significantly from those of all other landraces and modern cultivars, whose GPC values were similar.

Antioxidant activity

Wheat antioxidant activity was assessed according to TPC and ORAC. The only significant difference was in the ORAC of wild and modern wheats, whereas there were no significant differences in TPC among the wild, landrace and cultivar groups. No significant differences for either parameter occurred between the wild taxa and their domesticated descendants (*T. boeoticum*/*T. monococcum*; *T. dicoccoides*/*T. dicoccon*; and *T. araraticum*/*T. timopheevii*). However, the wild diploid taxa had significantly more TPC than *T. dicoccon* and *T. timopheevii*. *Triticum zhukovskyi* stood out with the highest TPC and ORAC. Fewer significant differences were found for ORAC than for TPC among the taxa. For both phenolic measurements, *T. zhukovskyi* showed the highest values (Fig. 3).

Yield parameters

Wild wheats had a significant lower TKW and grain weight per plant compared to the landrace taxa and modern cultivars (see Supporting information, Fig. S1). In comparison, the TKW was higher for the tetraploid *T. dicoccoides* and *T. araraticum* than for the wild dipoid wheats *T. boeoticum* and *T. urartu*, but, for the grain weight per plant, no difference among the wild wheats was observed. *Triticum zhukovskyi* had the lowest grain weight per plant and together with *T. monococcum* it was in the same range as the wild taxa.

Relationship between grain quality traits, yield parameters and taxa

The micronutrients were significantly positively correlated with the phytate storage molecule, but the negative correlation between the Phyt:Fe ratio and phytate was non-significant. Fe and Zn and GPC were also significantly positively correlated. The grain weight per plant was significantly negatively correlated with the micronutrients and GPC, but Zn was not correlated with the TKW. ORAC was the only grain quality parameter that was independent of the grain weight per plant. TPC was significantly positively correlated with Zn and ORAC (Table 2).

The PCA explained 76.4% of the variation in the dataset, with most of the variation derived from principal component PC 1 (56.7%) and 19.7% contributed by PC2. PC1 was associated with Fe, Zn, GPC and P, and PC2 was associated with grain weight per plant and Zn. The wild taxa were clustered, whereas the landraces were more scattered. *Triticum zhukovskyi* was located distantly from the other landraces, and *T. monococcum* was located between the landrace and wild taxa (Fig. 4). Furthermore, the modern cultivars and their landraces were proximate to each other. The wild taxa were associated with the Fe, phytate and GPC grain quality parameters, whereas the modern cultivars and the landraces (*T. durum*, *T. aestivum* and *T. timopheevii*) were clustered closer to the grain weight per plant and TKW yield parameters.

DISCUSSION

Alteration of grain quality through domestication is reflected in differences between wild wheats and their domesticated landrace taxa

Wheat domestication was accompanied by numerous morphological and genetic changes. To assess alterations in grain quality

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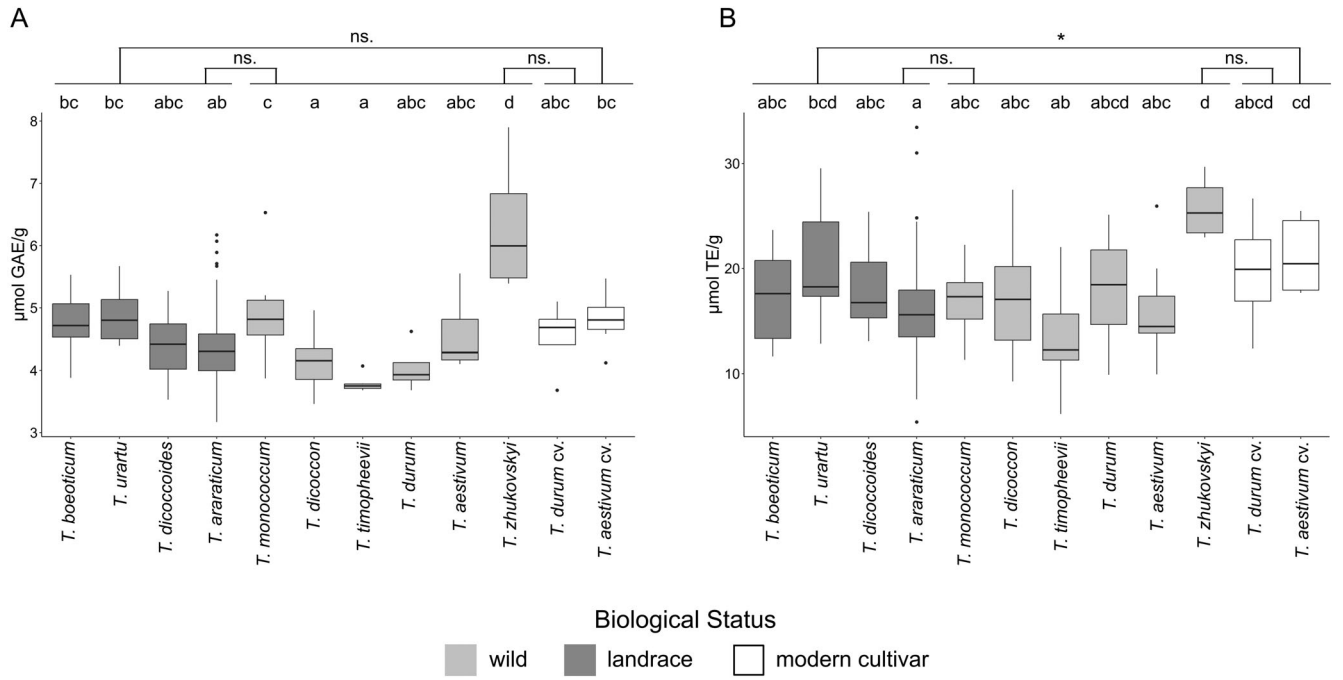

Figure 3. Antioxidant activity of wild taxa, landraces and modern cultivars. (A) Total phenolic content [µmol gallic acid equivalents (GAE) g^{-1}] of wild taxa, landraces and modern cultivars. (B) Oxygen radical absorption capacity [µmol Trolox equivalents (TE) g^{-1}] of wild taxa, landraces and modern cultivars. Asterisks indicate significant differences between groups according to their biological status (*$P < 0.05$: **$P < 0.01$; ***$P < 0.001$); ns, no significant difference. Different letters indicate significant differences among taxa ($P < 0.05$).

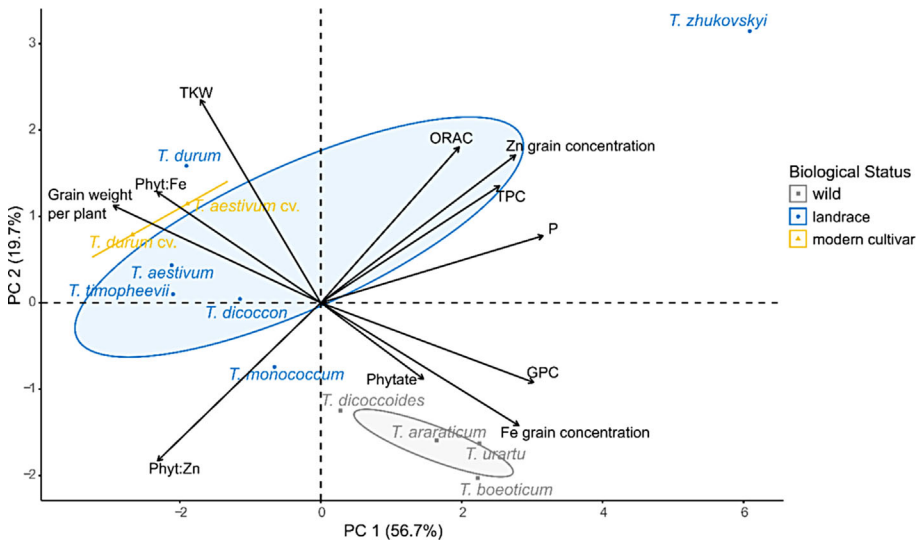

Figure 4. Principal component analysis of taxa and grain quality and yield parameters of thousand kernel weight (TKW) and grain weight per plant. Confidence ellipses are shown for biological status. TPC denotes total phenolic content and ORAC denotes oxygen radical absorbance capacity.

between wild wheats, their domesticated landraces and modern wheat cultivars, a diverse set of *Triticum* taxa was sown in the same location and year.

The total precipitation was 613.7 mm during cultivation. Thus, the plants had sufficient water.

Fe and Zn are essential micronutrients for human health and bodily functions.[4,5] As a staple food, wheat can provide required amounts of these elements. Grain Fe concentrations in modern bread wheat cultivars range from 25.8 mg kg^{-1},[38] to 29 mg kg^{-1},[39] up to 56.5 mg kg^{-1}.[12] The range for grain Zn concentrations is 20 mg kg^{-1},[39] to 36.8 mg kg^{-1},[38] up to 53.3 mg kg^{-1}.[12] Comparatively, grain concentrations of our modern bread wheat cultivars were in the upper range for Fe (46.6 mg kg^{-1}) and Zn (44.4 mg kg^{-1}). However, micronutrient concentrations are reportedly higher in wild wheats than in landraces and modern cultivars,[6,9,16,39,40] as confirmed in the present study.

Studies have found that *T. dicoccoides* is a promising candidate for Zn biofortification, given its high genetic diversity.[16,39] Our results confirmed the diversity of *T. dicoccoides* for grain Zn concentration (coefficient of variation: 31.6%), but we found it had

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the lowest grain Zn concentration among the wild wheats. *Triticum araraticum* and *T. boeoticum* showed the highest grain Zn concentrations of 60.1 mg kg^{-1} and 57.6 mg kg^{-1}, respectively. The grain Fe accumulation in *T. boeoticum*, *T. dicoccoides* and *T. araraticum* wild taxa was higher than that of *T. monococcum*, *T. dicoccon* and *T. timopheevii* landrace taxa (Fig. 1B). This pattern for grain Zn accumulation was found only in the domestication of *T. boeoticum* to *T. monococcum* and of *T. araraticum* to *T. timopheevii* (Fig. 1D). Therefore, we posit that domestication influenced the ability of grains to accumulate micronutrients.

The health-promoting functions of micronutrients are further determined by their bioavailability. In the present study, the molar ratio of the phytate and respective micronutrient served as a proxy for bioavailability. Good bioavailability was indicated by a Phyt:Fe molar ratio of < 1 for Fe,[41] and a Phyt:Zn ratio of < 15, but optimally < 5 for Zn.[42] The highest Fe bioavailability was found in the diploid wild taxa, *T. boeoticum* (Phyt:Fe: 10.3) and *T. urartu* (Phyt:Fe: 10.6). The lowest bioavailability was found for *T. durum* cv. (Phyt:Fe: 23.3) and *T. durum* (Phyt:Fe: 19.8), which corresponds to other Phyt:Fe ratios measured in *T. durum* cultivars (Phyt:Fe: 16.3–29.60).[43] The estimated Fe bioavailability in bread wheat ranges between 15.5 and 31.3,[44] whereas our *T. aestivum* landraces and bread wheat modern cultivars showed Phyt:Fe molar ratios of 18.6 and 14.5, respectively. Reported estimates of Zn bioavailability in durum wheat range between 17.4 and 23.6,[43] 23.9, and 41.4,[45] and between 49 and 116,[46] and, for bread wheat, the estimated range is 29–178.[46] Therefore, our values lie within the lower range. The differences in the Phyt:Fe and Phyt:Zn molar ratios were highest for *T. boeoticum* and *T. monococcum*, whereas *T. araraticum* and *T. timopheevii* showed almost identical values. The Phyt:Fe ratio increased slightly through domestication from *T. dicoccoides* to *T. dicoccon*, but that for Phyt:Zn decreased. Therefore, an alteration in bioavailability was observed only for domestication from *T. boeoticum* to *T. monococcum*.

Our data showed that domestication clearly affected the grain micronutrient concentration and slightly affected the estimated micronutrient bioavailability. The latter observation is preliminary because previous studies have not directly compared phytate concentrations in wild wheats and domesticated landraces grown in the same field. However, a focus on Phyt:micronutrient ratios for single genotypes could reveal promising wild genotypes for *de novo* domestication or improved biofortification of wheat.

The GPC is an important parameter that determines the end-use quality of wheat flour. In bread wheat, its value ranges between 7% and 18%[47] and is highly dependent on the growing environment.[17] In the present study the plants were grown as singly without fertilizer application during cultivation. Thus, they did not experience intraspecific competition for nutrients, as would occur in an agronomic field situation. In the present study, the modern cultivars had the lowest GPC compared with wild and landrace taxa (Fig. 2; see also Supporting information, Table S3), but the GPC of *T. aestivum* was slightly higher than the values recorded in other studies.[17,19] *Triticum monococcum* had an elevated GPC,[48,49] but, for *T. durum*, *T. dicoccon* and *T. dicoccoides*, the measured GPC was in the range of values reported in the literature.[49-51] In general, wild wheats and landraces are known to have higher GPC.[15,50-51] One *T. urartu* genotype showed 28% protein content.[52] The five *T. urartu* genotypes analyzed in the present study showed lower GPC (192–229 g kg^{-1}). Similar to *T. dicoccoides*, the GPC values of *T. boeoticum* and *T. araraticum* were significantly higher than those of their domesticated descendants (Fig. 2). This observation can be explained by the generally negative relationship between yield and GPC,[19,53] which our data confirmed (Table 2). These results reveal the influence of domestication on grain quality. We can thus conclude that grain quality was altered during domestication and that decreased GPC accompanied landrace development through the wild ancestor's domestication.

Some studies suggest that domesticated relatives of wheat have health-promoting effects, such as higher antioxidant capacity and phenolic content compared to wild ancestors,[20,52] although this assumption has been challenged.[22,54-56] However,

Table 2. Correlation matrix for the grain quality parameters ($N = 250$)

	Fe grain conc.	Zn grain conc.	Phyt: Fe	Phyt: Zn	P	Phytate	GPC	TPC	ORAC	TKW	Grain weight per plant
Fe grain conc.		0.39	−0.89	−0.32	0.49	0.28	0.53	0.07	0.02	−0.3	−0.37
Zn grain conc.	***		−0.28	−0.89	0.56	0.30	0.45	0.20	0.09	0.00	−0.34
Phyt:Fe	***	***		0.26	−0.38	−0.04	−0.44	−0.07	0.01	0.27	0.32
Phyt:Zn	***	***	***		−0.39	−0.11	−0.31	−0.09	0	−0.06	0.25
P	***	***	***	***		0.31	0.44	0.06	0.07	−0.21	−0.31
Phytate	***	***	NS	*	***		0.50	−0.05	−0.06	−0.26	−0.31
GPC	***	***	***	***	***	***		0.05	−0.08	−0.36	−0.60
TPC	NS	**	NS	NS	NS	NS	NS		0.46	−0.24	−0.15
ORAC	NS	NS	***	NS	NS	NS	NS	***		−0.09	0.07
TKW	***	NS	***	NS	**	***	***	***	NS		0.59
Grain weight per plant	***	***	***	***	***	***	***	*	NS	***	

Note: The upper triangle (above the black rectangles) shows the Pearson's correlation coefficients. The lower triangle (below the black rectangles) shows the significance of the correlations.
Abbreviations: conc, concentration; NS, not significant; Phyt, phytate; Fe, iron; Zn, zinc; P, phosphorus; GPC, grain protein content; TPC, total phenolic content; ORAC, oxygen radical absorption capacity; TKW, thousand kernel weight.
*$P < 0.05$;
** $P < 0.01$;
*** $P < 0.001$.

these latter studies focused primarily on domesticated Emmer and Einkorn wheats. Thus, data for wild wheats are scarce. Our observations for both parameters confirmed the non-superiority of both domesticated and wild wheats relative to modern wheats (Fig. 3). The TPC and ORAC of wild wheats and their domesticated descendants were almost the same (Fig. 3). Therefore, a domestication effect was not observed for this trait. Notably, the modern wheat cultivars showed the highest TPC and ORAC compared to the two other wheat groups of differing biological status. Hence, no negative breeding effect regarding antioxidant properties was identified.[22]

We found that the change from a wild to a domesticated plant caused alterations in several traits (Fig. 4), thus confirming our hypothesis that domestication modifies wheat grain quality. The PCA analysis grouped the wild wheats more distantly from the landrace taxa compared with the distance of the landrace taxa from the modern cultivars. Therefore, quality traits were evidently influenced during domestication. They were also negatively correlated with yield-related traits, such as TKW and grain weight per plant (Table 2), which could cause changes in grain quality parameters. However, other reasons could be unintended loss of grain quality traits during the domestication and selection history. In a *de novo* domestication project, those drivers would be bypassed. Furthermore, this approach could be an option in the future for eliminating unwanted traits during the domestication and selection process. Because the grain micronutrient concentration, phytate molecular ratio and GPC of wild wheats were higher than those of the landraces, they could be promising candidates for *de novo* domestication.

The GGAA wheat lineage harbors untapped diversity for grain quality improvement

GGAA wheats have received little attention, but this situation is gradually changing.[8,57] Moreover, the few studies on GGAA wheats have mainly examined cytogenetics or genetic diversity, although they may also have beneficial grain quality traits. Of the GGAA wheats in the present study, *T. araraticum* and *T. zhukovskyi* stood out, whereas *T. timopheevii* did not show superior characteristics compared with other landrace taxa. Only a few studies have examined micronutrients in *T. araraticum*. One study reported 23.1–59.1 mg kg^{-1} of Fe and 19.3–30.5 mg kg^{-1} of Zn in *T. araraticum*,[58] whereas ranges of grain Fe and Zn concentrations in *T. araraticum* genotypes in the present study were 44–115.8 mg kg^{-1} and 38.5–85.3 mg kg^{-1}, respectively. Therefore, *T. araraticum* genotypes had very high grain Fe concentrations. Of the landrace taxa, *T. zhukovskyi* was an exception, evidencing high grain Fe and Zn concentrations (Fig. 1B,D). Hence, among the GGAA wheats *T. araraticum* and *T. zhukovskyi* demonstrated high grain micronutrient concentration.

Our analysis of the phytate:micronutrient molar ratio showed that the Phyt:Fe ratios of *T. araraticum*, *T. timopheevii* and *T. zhukovskyi* were almost equal (Fig. 1C,E). The Phyt:Zn ratios showed high estimated Zn bioavailability for *T. zhukovskyi* and similar bioavailability for *T. araraticum* and *T. timopheevii*. However, for single genotypes, Phyt:Fe ratios ranged between 7.2 and 7.9 for *T. araraticum* genotypes, whereas Phyt:Zn ratios indicated that only *T. zhukovskyi* showed moderate bioavailability (8.6). *T. araraticum* had the highest Phyt:Zn ratio (16.7) among the wild taxa. Although *T. araraticum* and *T. zhukovskyi* were the most promising taxa given their estimated high Fe bioavailability, none of the molar ratios indicated good bioavailability as requested.[41,42] Yet it was better than in the modern wheats and our findings therefore demonstrate that GGAA wheats are potential sources for biofortification. *Triticum araraticum* harbors high potential for Fe biofortification and is a potential candidate for *de novo* domestication. *Triticum zhukovskyi* showed elevated grain micronutrient accumulation and estimated bioavailability. Adding GGAA-derived wheats with higher estimated Fe bioavailability to a wheat-dominated diet could enhance nutrition, helping to prevent malnutrition. Furthermore, among the wild wheats, *T. araraticum* is suitable for cultivation and exhibits favorable yield parameters.[32] Therefore, *T. araraticum* and *T. zhukovskyi* should be considered as sources for developing more nutritious wheat cultivars.

The wild wheats, including *T. araraticum*, showed high GPC values. *Triticum zhukovskyi* was again exceptional among the landrace taxa, with the highest GPC (239 g kg^{-1}) (Fig. 2). This finding contrasts with that of a previous study,[21] which reported a GPC of 16.92% in 'Far 75', a *T. zhukovskyi* accession and 20.1% in 'Lonigo', a *T. timopheevii* accession. GPC was lower in our *T. timopheevii* genotypes, ranging between 167 g kg^{-1} and 173 g kg^{-1}. However, not only its protein content, but also its composition is important for baking and cooking quality. A previous study[21] analyzed the gluten quality of *T. timopheevii* and *T. zhukovskyi* and identified high gluten content but low gluten quality. Therefore, their usage was recommended for flatbread, biscuits and pasta. Given their high GPC, the wild wheats could also show the same pattern. Therefore, future studies should assess their gluten quality to determine potential end uses. Furthermore, Einkorn wheat, *T. durum* and *T. dicoccon* proteomes show high diversity and lower allergenic potential.[59,60] Similar analyses should be conducted for the GGAA wheats because it remains unclear whether elevated GPC in *T. araraticum*, *T. timopheevii* and *T. zhukovskyi* favors high end-use quality.

Triticum zhukovskyi stood out for its antioxidant potential, with very high TPC and ORAC (Fig. 3). These values were the highest among those of the GGAA wheats, and of all wheat taxa in our study. Therefore, this taxon should be investigated further.

In sum, *T. araraticum* and *T. zhukovskyi* showed beneficial grain quality characteristics. We can therefore conclude that they harbor beneficial grain quality properties for enhancing nutrition.

Identification of suitable candidates for *de novo* domestication

The wild taxa showed clear associations with favorable grain quality attributes, such as grain micronutrient concentration (Fig. 4). Our analysis showed that wild wheats are distinguished mainly by their micronutrient accumulation, bioavailability and GPC. However, as discussed above, a more thorough investigation of GPC is required to draw conclusions regarding grain quality for different types of use. Therefore, our recommendations for further use of genotypes will focus on micronutrient bioavailability. Two *T. araraticum* genotypes (91 and 139) (see Supporting information, Table S1) showed the highest Fe bioavailability (Phyt:Fe ratios: 7.5 and 7.2, respectively) and high Zn bioavailability (Phyt:Zn ratios: 12.2 and 12.9, respectively). *Triticum boeoticum* (genotype no. 27; Phyt:Zn = 11.5; Phyt:Fe = 9.5) and *T. araraticum* (genotype no. 203 [Phyt:Zn = 11.7; Phyt:Fe = 9.7] and genotype no. 131 [Phyt:Zn = 11.8; Phyt:Fe = 9.6]) can be recommended for their Zn bioavailability.

Although *T. araraticum* has not been extensively researched for grain quality traits, our data showed that this species can be a good source for grain quality improvement. To confirm these findings, favorable genotypes should be grown in different

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environments in future experiments. Performing *de novo* domestication via genome editing will reveal whether unintended loss of beneficial alleles is bypassed or whether grain quality changes with an increase in yield-related traits. We previously proposed genes linked to cultivation, harvest and yield for wild wheat *de novo* domestication.[32] When assessing differences in grain quality between wild and domesticated taxa, the proposed genes should not be edited all at once; rather, a stepwise strategy should be adopted to determine whether any changes in grain quality occur when domestication genes are modified.

Apart from the wild taxa, *T. zhukovskyi* showed outstanding grain quality. Differing from the other domesticated wheats, it was clustered along PC1, which was positively associated with the analyzed grain quality parameters (Fig. 4). The two genotypes analyzed here showed high bioavailability for both micronutrients and high antioxidant potential. Thus, investigating *T. zhukovskyi* as a favorable taxon for achieving enhanced grain quality holds promise. As *T. zhukovskyi* has already undergone domestication, breeding efforts can focus on achieving higher yield, cultivation management, and/or quality improvement.

CONCLUSIONS

To the best of our knowledge, the present study is the first to analyze and compare the grain quality of different wild taxa, wheat landraces and modern wheat cultivars grown in the same environment. A decrease in grain micronutrient concentration and GPC accompanied the transition from wild to domesticated wheat. In terms of bioavailability, wild wheats have an advantage over domesticated wheats and are therefore suitable candidates for *de novo* domestication. We have reported novel data on the grain quality of *T. araraticum* and its GGAA relatives. *T. araraticum* and *T. zhukovskyi* showed favorable grain quality characteristics and should be further explored. However, other relatable traits, such as starch content, gluten composition and other phytochemical characteristics, require further exploration. Because of its superior grain quality, *T. araraticum* appears to be a sound choice for *de novo* domestication to avoid deleterious domestication effects over more than 10 000 years of domestication and selection. We also recommend establishing a dedicated *T. zhukovskyi* breeding program or conventional bread wheat improvement programs with *T. zhukovskyi* and *T. araraticum* as donors for favorable grain quality traits.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

FZ planned the laboratory work, prepared the samples, carried out experiments, collected and analyzed data, created the figures and tables, and structured and wrote the manuscript. BK contributed to and edited the manuscript. HÖ provided the plant material, and contributed to and edited the manuscript. SP edited the manuscript. MF conceived the project and contributed to and edited the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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