Institute of Agronomy and Plant Breeding I

Department of Agronomy and Crop Physiology

Research Centre for Biosystems, Land Use and Nutrition (iFZ)

Justus Liebig University Giessen

Prof. Dr. Michael Frei

Exploring the domestication and agricultural potential of wheat wild relatives

INAUGURAL DISSERTATION

Submitted in fulfillment of the requirements for the degree

Doctor agriculturae (Dr. agr.)

to the

Faculty of Agricultural Sciences, Nutritional Sciences,

and Environmental Management

Justus Liebig University Giessen

Submitted by

Frederike Zeibig

Giessen, May 2024

With permission by the

Faculty of Agricultural Sciences, Nutritional Sciences,

and Environmental Management

Justus Liebig University Giessen

Date of thesis disputation: 22nd July 2024

Examination committee:

Head of examination committee: Prof. Dr. Marc F. Schetelig

1st Examiner (supervisor): Prof. Dr. Michael Frei

2nd Examiner (co-supervisor): Prof. Dr. Agnieszka Golicz

3rd Examiner: Prof. Dr. Jakob Santner

4th Examiner: Prof. Dr. Sylvia Schnell

List of Content

Abbreviations

Ae.	*Aegilops*
AP2	*APTETALA2*
BE	Base Editing
bp	base pair
Btr	*Brittle rachis*
CWRs	Crop Wild Relatives
DSB	Double-Strand Breaks
GA	Gibberellin
GPC	Grain Protein Content
HDR	Homology-Directed Repair
HI	Harvest Index
Fe	Iron
FT	*FLOWERING LOCUS*
NDVI	Normalized Difference Vegetation Index
NHEJ	Non-Homolologous End Joining
O.	*Oryza*
ORAC	Oxygen Radical Absorbance Capacity
PE	Prime Editing
PEG	Polyethylene Glycol
Rht	Reduced Height
RUE	Radiations Use Efficiency
S.	*Solanum*
sgRNA	single guide RNA
SNP	Single Nucleotide Polymorphism
T.	*Triticum*
TKW	Thousand Kernel Weight
TPC	Total Phenolic Content
WAPO	*WHEAT ORTHOLOG OF APO*
Zn	Zinc

List of Figures

Appendix – List of Figures

List of Tables

Appendix – List of Tables

Summary

Domestication laid the foundation of agriculture by modifying plants to suit human needs. This process and subsequent breeding induced genetic bottlenecks in domesticated plants leading to an altered genetic diversity. However, ancient diversity has been preserved in crop wild relatives (CWRs). Wheat (*Triticum aestivum* L.; *Triticum durum* Desf.) is a staple crop with a long breeding history starting with domestication. Its evolutionary diversity includes diploid wild *Triticum urartu* and *Triticum boeoticum*, diploid domesticated *Triticum monococcum*, tetraploid wild *Triticum dicoccoides* and domesticated *Triticum dicoccon* from the Emmer lineage and the GGAA wheats wild *Triticum araraticum*, domesticated *Triticum timopheevii* and hexaploid domesticated *Triticum zhukovskyi*. These taxa display a vital resource of diversity and harbor beneficial traits for biotic and abiotic stress tolerance as well as for improved grain quality. To take advantage of these favorable attributes, crossing of modern wheat with a relative is commonly used for incorporating the target trait. A new approach is *de novo* domestication - the modification of domestication genes in CWRs via genome editing - which modifies the wild relative in a cultivable crop itself while retaining the untapped genetic diversity and beneficial traits. For a successful *de novo* domestication, suitable taxa and genotypes for the target environment, target traits for modification and unique and beneficial traits like elevated grain quality need to be determined. The aim of this thesis was to explore the wild *Triticum* taxa for their agricultural and domestication potential and thus to pave a way for a *de novo* domestication of wild wheat with beneficial grain quality.

Therefore, the beneficial potential of wheat wild relatives for grain quality improvement and the change of grain quality during evolution and domestication was reviewed. The wild wheats were proposed as potential candidates for a *de novo* domestication. To evaluate the adaptability of wild wheats to the central European climate and identify target traits for a *de novo* domestication, 111 wild wheat genotypes and for comparison 38 landrace genotypes and six modern wheat cultivars were grown in a field at the research station of the University of Giessen in 2020/21. Out of these 89 wild wheats, 30 landraces and six modern wheat cultivars were assessed for their grain Iron (Fe) and Zinc (Zn) concentration and estimated bioavailability, Grain Protein Content (GPC) and antioxidant potential to identify changes in grain quality during domestication and to select wild wheats with superior grain quality for a *de novo* domestication.

The wild wheats showed a good adaptability to the central European climate indicated by similar Normalized Difference Vegetation Index (NDVI) and anthesis to modern wheats. Shattering and tight glumes impaired harvest and were proposed as target traits for a *de novo* domestication. Furthermore, significant lower yields were observed in wild wheats compared to landraces and modern wheat cultivars. Therefore, yield traits were suggested to be modified in a *de novo* domestication. Possible target genes for modifying the undesirable traits were also identified. The grain quality analysis revealed high grain Fe and Zn concentration accompanied by a good estimated bioavailability for the respective micronutrients and a high GPC in wild wheats compared to the landrace taxa and modern cultivars. Among the wild wheats, *T. araraticum* from the GGAA wheats was outstanding for the grain quality traits. Therefore, four *T. araraticum* genotypes were selected as potential candidates for a *de novo* domestication. Analyzing the grain quality data together with the phenotypic and yield data, they were identified as suitable additions to the wheat cropping system. Therefore, different cultivation scenarios for *de novo* domesticated *T. araraticum* were outlined to ensure acceptance and application in future cropping systems.

The identification of suitable *T. araraticum* genotypes with beneficial grain quality traits prepared the first step towards a *de novo* domestication of the wheat wild relative *T. araraticum*. Thus wild wheats harbor a potential for *de novo* domestication that can be leveraged in the future to improve the diversity of the cropping system.

Zusammenfassung

Die Domestikation war ein zentraler Prozess, der zur Entstehung der Landwirtschaft beitrug, indem Pflanzen an die Bedürfnisse des Menschen angepasst wurden. Dieser Prozess und die anschließende Züchtung führten zu genetischen Flaschenhälsen innerhalb der domestizierten Pflanzen, wodurch sich die genetische Vielfalt veränderte. Die ursprüngliche Diversität ist jedoch in den wilden Verwandten der Kulturpflanzen erhalten geblieben. Weizen (*Triticum aestivum* L., *Triticum durum* Desf.) ist ein Grundnahrungsmittel mit einer langen Züchtungsgeschichte, die bereits mit der Domestikation begann. Die evolutionäre Vielfalt von Weizen umfasst die diploiden Wildweizen *Triticum urartu* und *Triticum boeoticum*, den diploiden domestizierten *Triticum monococcum*, den tetraploiden Wildweizen *Triticum dicoccoides* und den domestizierten *Triticum dicoccon* aus der Emmer Linie sowie aus der Gruppe der GGAA-Weizen den wilden *Triticum araraticum*, den domestizierten *Triticum timopheevii* und den hexaploiden domestizierten *Triticum zhukovskyi*. Diese Taxa stellen eine wichtige Ressource der Vielfalt dar und beherbergen vorteilhafte Eigenschaften bezüglich biotischer und abiotischer Stresstoleranz sowie für eine verbesserte Kornqualität. Zur Nutzung dieser vorteilhaften Eigenschaften wird moderner Weizen mit einem wildem Verwandten gekreuzt, um das gewünschte Merkmal in den Weizen einzubringen. Ein neuer Ansatz ist die *de novo* Domestikation – die Modifikation von Domestikationsgenen in wilden Verwandten mittels Genome Editing – wodurch der wilde Verwandte selbst genutzt wird und in eine Kulturpflanze umgewandelt wird. Hierbei bleibt die unberührte genetische Vielfalt sowie die vorteilhaften Eigenschaften der wilden Verwandten erhalten. Für eine erfolgreiche *de novo* Domestikation müssen geeignete Taxa und Genotypen für die Zielumgebung, die zu verändernden Eigenschaften und einzigartige und vorteilhafte Merkmale, wie gute Kornqualität, ermittelt werden. Ziel dieser Arbeit war es, das Potenzial der *Triticum* Taxa bezüglich ihres Anbau- und Domestikationspotenzials zu evaluieren und somit den Weg für eine *de novo* Domestikation von Wildweizen mit guten Nährstoffeigenschaften zu ebnen.

Hierzu wurde das Potenzial der wilden Verwandten des Weizens für die Verbesserung der Kornqualität sowie die Veränderung der Kornqualität während der Evolution und der Domestikation untersucht. Die wilden Weizenarten wurden als potenzielle Kandidaten für eine *de novo* Domestikation identifiziert. Um das Anpassungspotenzial der Wildweizenarten an das mitteleuropäische Klima zu evaluieren und um Zielmerkmale für eine *de novo* Domestikation zu bestimmen, wurden in den Jahren 2020/21 im Rahmen eines Feldversuches auf der Forschungsstation der Universität Gießen 111 Wildweizen Genotypen und zum Vergleich 38 Landrassen Genotypen sowie sechs moderne Weizensorten angebaut. Von diesen Genotypen wurden 89 Wildweizen, 30 Landrassen und sechs moderne Weizensorten hinsichtlich der Eisen- und Zinkkonzentration im Korn sowie der geschätzten Bioverfügbarkeit der beiden Mikronährstoffe, des Kornproteingehaltes und des antioxidativen Potenzials analysiert, um Veränderungen in der Kornqualität während der Domestikation zu identifizieren und Wildweizengenotypen mit positiver Kornqualität für eine *de novo* Domestikation auszuwählen.

Die Wildweizenarten zeigten eine gute Anpassungsfähigkeit an das mitteleuropäische Klima, was sich in einem ähnlichen Normalized Difference Vegetation Index (NDVI) und Blütezeitpunkt im Vergleich zu modernen Weizensorten zeigte. Spindelbrüchigkeit und feste Spelzen beeinträchtigten die Ernte und wurden als Zielmerkmale für eine *de novo* Domestikation identifiziert. Im Vergleich der Wildweizenarten zu den Landrassen und modernen Weizensorten wurden signifikant geringere Erträge festgestellt, weshalb die Modifikation von Ertragsmerkmalen im Zuge einer *de novo* Domestikation empfohlen wurde. Weiterhin wurden mögliche Gene zur Modifikation der ungewünschten Eigenschaften identifiziert. Die Analyse der Kornqualität ergab eine hohe Eisen- und Zinkkonzentration in den Körnern verbunden mit einer guten geschätzten Bioverfügbarkeit der beiden Mikronährstoffe und einem hohen Kornproteingehalt im Wildweizen im Vergleich zu den Landrassen und modernen Weizensorten. Innerhalb der Wildweizenarten zeigte *T. araraticum* aus der Gruppe der GGAA-Weizen herausragende Eigenschaften für die Kornqualität. Aufgrund dessen wurden vier *T. araraticum* Genotypen als potentielle Kandidaten für eine *de novo* Domestikation ausgewählt. Die Analyse der Kornqualitätsdaten zusammen mit den phänotypischen Merkmalen und den Ertragsdaten ergab, dass die vier *T. araraticum* Genotypen eine geeignete Ergänzung im Weizenanbau darstellen könnten. Daher wurden verschiedene Anbauszenarien für *de novo* domestizierten *T. araraticum* skizziert, um die Akzeptanz und Anwendung in zukünftigen Anbausystemen zu gewährleisten.

Die Identifikation von geeigneten *T. araraticum* Genotypen mit vorteilhaften Kornqualitätseigenschaften bereitete den ersten Schritt für eine *de novo* Domestikation des wilden Weizenverwandten *T. araraticum*. Somit beherbergen wilde Weizenverwandte das Potenzial für eine *de novo* Domestikation, das in Zukunft genutzt werden kann, um die Vielfalt des Anbausystems zu verbessern.

1 General Introduction

1.1 Domestication

1.1.1 The process of domestication

Domestication of crops enabled our modern agricultural system. Plant domestication described the evolution of wild plants into cultivated crops that meet human needs (Salamini *et al.*, 2002; Doebley *et al.*, 2006; Bartlett *et al.*, 2023). This gradual process altered morphological and physiological traits of plants caused by human selection (Hancock, 2005; Stetter *et al.*, 2017; Hebelstrup *et al.*, 2023). Selection was based on phenotypic evaluation (Doebley *et al.*, 2006; Jian *et al.*, 2022) and included conscious and unconscious selection (Zohary, 2004). The evolution of domestication encompassed three phases, which led to the emergence of crops completely dependent on human (Zohary, 2004; Doebley *et al.*, 2006; Zeder, 2006). First annual plants were collected from their natural habitats, followed by cultivation, which comprised a systematic planting of wild plants in a field. Cultivation was an essential phase because wild plants underwent a repetitive cycle of sowing, harvesting, and replanting that contributed to the accumulation of preferred genotypes. This process eventually led to the domestication phase where plants with favorable traits were raised (Weiss *et al.*, 2006). Therefore, domestication was a continuous and time-intensive process (Zsögön *et al.*, 2022).

1.1.2 Crop and genetic diversity during domestication

Similar traits were targeted and modified during domestication for the same group of plants across different environments, time and independent human populations. The resulting phenotype was defined as the domestication syndrome (Doebley *et al.*, 2006; Ross-Ibarra *et al.*, 2007; Kantar *et al.*, 2017). For grasses, the most common traits were the loss of seed shattering, modification of seed size and dormancy (Doebley *et al.*, 2006; Kantar *et al.*, 2017; Stetter *et al.*, 2017; Fernie & Yan, 2019). Previous studies assumed that the domestication phenotype evolved from only a few genes (Gross & Olsen, 2010), whereas recently growing evidence for a contribution of numerous loci was built (Stetter *et al.*, 2017; Curtin *et al.*, 2021). Domestication genes were derived from a loss or gain of function mutation, *de novo* mutation and enhancement or decrease of gene expression (Meyer & Purugganan, 2013; Østerberg *et al.*, 2017; Smýkal *et al.*, 2018; Jian *et al.*, 2022). Yet domestication was a complex process that altered genetics and molecular pathways. A high selection pressure on domestication genes for fixation in the newly derived population led to a reduced genetic diversity in their proximity (Smýkal *et al.*, 2018). A change in diversity not only occurred on the genetic level but also on species level. Among all wild plants, only a few species were domesticated thus narrowing (genetic) diversity in the agricultural cropping system (Ladizinsky, 1985; Doebley *et al.*, 2006; Stetter *et al.*, 2017). Characteristic of these crops, like the staples wheat

(*Triticum aestivum* L.), rice (*Oryza sativa* L.) and maize (*Zea mays* L.), was an early domestication that enabled a good adaption to agro-environmental conditions. Therefore, the cultivation system of these crops was continuously improved (Milla & Osborne, 2021). The combined effect of selection for species and further selection of genotypes and traits within these populations narrowed the genetic diversity starting with domestication and subsequently continued with breeding effort. This process was referred to as the genetic bottleneck (Tanksley & McCouch, 1997).

Crop wild relatives (CWRs) still harbor a reservoir for high genetic diversity. Furthermore, CWRs evolved individually and adapted to marginal environments that led to the emergence of adaptive alleles (Stetter *et al.*, 2017). This untapped genetic diversity can be used for breeding for biotic and abiotic stress tolerance as well as improved nutritional value. The process of introgression of beneficial wild genes into modern elite crops is tedious due to linkage drag of wild unfavorable traits that hamper the elite cultivar’s performance and need to be eliminated via backcrossing (Sharma *et al.*, 2021; Sukumaran *et al.*, 2022). Furthermore, breeding for stress tolerance requires multiple genes due to its quantitative nature. Incorporation of each gene into the modern crop for improvement is difficult (Sukumaran *et al.*, 2022). Therefore using the CWRs as a crop itself and modifying it for cultivation is another approach, which could also re-introduce diversity on species level.

1.2 *De novo* domestication

De novo domestication is the modification of domestication genes in wild plants or CWRs (Fernie & Yan, 2019; Khan *et al.*, 2019; Fernie *et al.*, 2021; Razzaq *et al.*, 2021; Bartlett *et al.*, 2023). The outcome is supposed to be a cultivable plant with maintained favorable attributes of the wild crop, e.g. stress tolerance and/or beneficial nutritional traits (Gasparini *et al.*, 2021; Razzaq *et al.*, 2021). This idea gained popularity by the emergence of genome editing and the discovery of CRISPR/Cas (Khan *et al.*, 2019; Razzaq *et al.*, 2021; Bartlett *et al.*, 2023). Yet, producing a *de novo* domesticated crop requires phenotyping, knowledge on the genetics of the CWR and molecular biology methods (Gasparini *et al.*, 2021; Yu & Li, 2022).

1.2.1 Phenotyping for de *novo* domestication

Crop wild relatives harbor a vast diversity not only from a genetic point of view, but also in number of accessions and phenotypes. Therefore, identification of the suitable material and candidate accession is crucial for a *de novo* domestication. The selection can be based on different considerations regarding the environment, unique advantageous traits and plant’s performance. The target environment can be an environment where the CWR thrives, such as a saline environment, a broader region with climatic requirements, a greenhouse or indoor farms (Brozynska *et al.*, 2016; Razzaq *et al.*, 2021; Bartlett *et al.*, 2023). Since CWRs are not as uniform as modern cultivars, testing in multiple locations is crucial to assess the CWRs' performance and confirm phenotypic observations (Sukumaran *et al.*, 2022). Furthermore, advantageous traits that justify the expense of a *de novo* domestication should be identified, such as an enhanced stress tolerance and enriched nutritional profile (Zsögön *et al.*, 2022).

1.2.2 Crop genomics for *de novo* domestication

Insight in the genetics of CWRs are required in order to perform a *de novo* domestication. Genomic tools for sequencing have improved for a higher accuracy which enables the availability of reference genomes. Furthermore, pangenomes are also used to cover the whole breadth of diversity for a distinct species (Gasparini *et al.*, 2021). For a *de novo* domestication, the sequenced CWR genome is crucial in order to identify the orthologues of known genes that control domestication traits (Østerberg *et al.*, 2017; Fernie & Yan, 2019). The closer the chosen CWR is related to a domesticated species, the easier the identification of orthologues is (Østerberg *et al.*, 2017). However it is also important to understand the whole function of the gene and its network (Fernie & Yan, 2019; Jian *et al.*, 2022). Knowledge on the gene and its alteration from wild to domesticated allele will determine the genome editing method of choice to modify the gene (Curtin *et al.*, 2021).

1.2.3 CRISPR/Cas and transformation for *de* novo domestication

CRISPR/Cas is a promising genome editing tool for plant improvement and accelerated crop breeding. The suitable CRISPR/Cas editing method depends on the target gene(s) and desired modification. CRISPR/Cas can cause DNA Double-Strand Breaks (DSB) that facilitate non-homolologous end joining (NHEJ) and homology-directed repair (HDR) pathways in the cell (Chen *et al.*, 2019; Khan *et al.*, 2019; Bartlett *et al.*, 2023). While NHEJ relies on a random composition of nucleotides for the reparation of DNA, which likely leads to a mutation of the target gene (Bartlett *et al.*, 2023), HDR requires a template DNA for repair (Chen *et al.*, 2019). Therefore, NHJE is well suited for introducing random mutations that result in a loss of function and HDR for a precise modification of point mutations, an insertion or replacement of sequences (Chen *et al.*, 2019; Capdeville *et al.*, 2023). Yet, a DSB is not required for Base Editing (BE) and Prime Editing (PE) (Cardi *et al.*, 2023). BE enables the precise modification of nucleotides in the target gene, by changing cytosine to thymine or adenine to guanine (Chen *et al.*, 2019; Capdeville *et al.*, 2023). PE can be used to modify sequences precisely (Capdeville *et al.*, 2023). The advantages of BE and PE over DSB methods are the more efficient modification that creates less undesired modifications (Chen *et al.*, 2019; Capdeville *et al.*, 2023). Hence, for a *de novo* domestication, all methods are suitable, but PE and BE are the best fit (Curtin *et al.*, 2021).

For a successfully genome edited plant, the CRISPR/Cas cassette needs to be delivered into the plant (Østerberg *et al.*, 2017). Different methods for this transformation have been developed. The most common one is the transformation via *Agrobacterium tumefaciens*, but Bombardement and Polyethylene Glycol (PEG) mediated transformation are suitable, too (Chen *et al.*, 2019; Ahmar *et al.*, 2023). However, for a broad use of CRISPR/Cas these transformation and regeneration protocols have to be genotype-independent while guaranteeing a high efficiency (Fernie & Yan, 2019, 2019; Cardi *et al.*, 2023). Development of suitable transformation and regeneration protocols for CWRs are a challenging prerequisite for *de novo* domestication (Khan *et al.*, 2019; Curtin *et al.*, 2021).

1.2.4 Successful examples of *de novo* domestication

The concept of *de novo* domestication was proven in wild and orphan crop species from the *Solanaceae* genus and a wild relative of rice.

Tomato (*Solanum lycopersicum* L.) is a widely studied model plant and a major crop plant with an established reference genome (The Tomato Genome Consortium, 2012). The closest wild relative of the domesticated tomato is *Solanum pimpinellifolium* L.. Therefore, it was a subject of a *de novo* domestication approach twice (Li *et al.*, 2018; Zsögön *et al.*, 2018). Main target traits were the modification of the plant growth habit and increased fruit size. For the former, *SELF-PRUNING* and *SELF-PRUNING 5G* were chosen for a determinant growth. Fruit size was targeted by the *FASCIATE*, *FRUIT WEIGHT 2.2*, *LOCULE NUMBER* and correlated genes *CLAVATA3* and *WUSCHEL*. Both studies used a multiplex CRISPR/Cas9 editing approach and created loss of function mutations in the targeted genes (Li *et al.*, 2018; Zsögön *et al.*, 2018). Zsögön *et al.* (2018) furthermore improved the nutritional quality by targeting *LYCOPENE BETA CYCLASE*, leading to a higher accumulation of the antioxidant lycopene in edited *S. pimpinellifolium* fruits. Thus, it proved that not only the wild phenotype, but also quality traits can be a subject in a *de novo* domestication. Lemmon *et al.* (2018) targeted the same traits and genes in the orphan crop *Physalis pruinosa*, which belongs to the *Solanaceae* family. Since it is a more distant relative of *Solanaceae* without a published reference genome, genome and transcriptome assembly was carried out prior to transformation. The genome edited plants showed an altered plant architecture, increased locule number and fruit mass, hence proofing that the concept of *de novo* domestication was also applicable to more distant related crops (Lemmon *et al.*, 2018). Another more distant related crop with a different ploidy level compared to its domesticate was the wild tetraploid *Oryza alta*, a wild relative of rice (Yu *et al.*, 2021). The selection of an *O. alta* genotype as a target for *de novo* domestication was based on its high transformation and regeneration efficiency as well as its biotic and abiotic stress tolerance (Yu *et al.*, 2021). Due to different genomes compared to *O. sativa* (AA) and ploidy levels, a high-quality genome was assembled differentiating for both sub-genomes.

Target traits for the *de novo* domestication of *O. alta* were shattering, awn length, plant height and grain length that were successfully modified by inducing frameshift mutations. Furthermore, base substitution was used to successfully *alter Ideal Plant Architecture 1* (*IPA1*) via a gain-of-function mutation (Yu *et al.*, 2021). This shows, that also taxa from a broader genepool can be used for a *de novo* domestication.

1.3 Wheat diversity and nutritional value

Wheat (*Triticum* spp.) together with rice and maize provides approximately 60% of human calories (Reynolds & Braun, 2022). Wheat accounts for 19% calories and 20% of proteins in the human diet (Langridge *et al.*, 2022) and thus ensures food security. Currently, wheat is cultivated in many different environments and breeding efforts created suitable cultivars for different target environments (Crespo-Herrera *et al.*, 2022). Wheat production is currently stable, but cultivation area is decreasing and annual productivity increases have stagnated. Due to a predicted population of 9.8 billion by 2050 this development imposes a threat to food security (Erenstein *et al.*, 2022). Furthermore, land degradation, pest and diseases and the effects of climate change are challenges for future wheat yields (Foulkes *et al.*, 2022). Yet, not only future wheat quantity is at risk, also the grain quality is impacted by elevated CO_2, heat, drought and salinity (Shewry, 2009; Zahra *et al.*, 2023). To combat these threats to food security, wheat relatives are an important resource since they harbor adaptive traits.

1.3.1 Wheat diversity

The *Triticum* genus is characterized by a wide diversity of taxa with different ploidy levels. The evolution of modern wheat was characterized by hybridization and polyploidization with members of the *Triticum* and *Aegilops* genus. Bread wheat, *T. aestivum*, has a hexaploid genome derived from the polyploidization event of a tetraploid wheat and *Aegilops tauschii*. Due to polyploidization and hybridization of different genera and domestication, the wheat gene pool harbors a broad and diverse germplasm. Wheat and its relatives are classified into primary, secondary and tertiary gene pools with respect to their genomic constitution, crossability and their degree of relationship (Tadesse *et al.*, 2019). The primary gene pool includes cultivars, landraces and wild species that are readily crossable with wheat and give rise to fertile hybrids (Tadesse *et al.*, 2019; Gupta *et al.*, 2020). This includes the wild Emmer wheat *Triticum dicoccoides* (Körn. ex. Aschers. & Graebner) Schweinf. ($2n = 4x = 28$, BBA^uA^u) and domesticated Emmer *Triticum dicoccon* Schübl. ($2n = 4x = 28$, BBA^uA^u) and *Triticum durum* Desf. ($2n = 4x = 28$, BBA^uA^u), the wild A genome donor *Triticum urartu* Thumanjan ex Gandilyan ($2n = 2x = 14$, A^uA^u) and *Ae. tauschii* (Tadesse *et al.*, 2019; Gupta *et al.*, 2020; King *et al.*, 2022). Taxa that have one or more homologeous genome with wheat are grouped in the secondary gene pool (Tadesse *et al.*, 2019; Gupta *et al.*, 2020). The GGAA wheats *Triticum araraticum* Jakubz. (2n = 4x = 28, GGA^tA^t) (wild) and *Triticum timopheevii* (Zhuk.) Zhuk (2x = 4n = 28, GGA^tA^t) (domesticated) belong to the secondary genepool as well as *Aegilops speltoides* Tausch (2n = 2x = 14, BB/GG) (Tadesse *et al.*, 2019; Gupta *et al.*, 2020; King *et al.*, 2022). The diploid domesticated Einkorn *Triticum monococcum* L. (2n = 2x = 14, AA) and its wild relative *Triticum boeoticum* Boiss (2n = 2x = 14, AA) are also part of the secondary genepool (Schoen *et al.*, 2024). Crossability of taxa from the secondary gene pool is more difficult and might require embryo rescue (Tadesse *et al.*, 2019; Gupta *et al.*, 2020). Special techniques for crossing wheat with taxa of the tertiary genepool are necessary due to the homoelogity of the chromosomes (King *et al.*, 2022). This criteria accounts for taxa of *Aegilops*, that were not involved in wheat evolution and other genera such as *Thinopyrum* and *Secale* (Tadesse *et al.*, 2019; Gupta *et al.*, 2020; King *et al.*, 2022).

A comprehensive overview of the domestication history of diploid, tetraploid and hexaploid wheats and their distribution is provided in Chapter 2 (Zeibig *et al*., 2022).

1.3.2 Wheat grain quality

As mentioned above, wheat mainly provides proteins and carbohydrates (Shewry, 2009; Igrejas *et al.*, 2020). Starch accounts for 70-80% of the carbohydrates in the endosperm of wheat grains (Shewry, 2009; Lafiandra *et al.*, 2014; Guzmán *et al.*, 2022). Wheat also provides a high amount of protein for human nutrition (Shewry, 2009). Wheat protein contains mostly gluten that confers the viscoelasticity of the dough and determines end use quality (Wieser, 2007; Shewry, 2009; Biesiekierski, 2017). Essential amino acids, dietary fibers, minerals and vitamins characterize wheat’s nutritional value (Shewry, 2009; Shewry & Hey, 2015; Igrejas *et al.*, 2020; Govindan *et al.*, 2022). Dietary fiber improves gut bacteria and prevents high peaks in blood glucose by a slower release (Lafiandra *et al.*, 2014; Govindan *et al.*, 2022). Mature wheat grains contain about 10% dietary fiber, mainly composed of cellulose, arabinoxylan and β-glucan (Lafiandra *et al.*, 2014; Shewry & Hey, 2015). Since they are a cell wall polysaccharides, they are prevalently located in the aleurone and pericarp (Shewry & Hey, 2015). The wheat bran is the storage site for B-vitamins provided by the wheat grain (Shewry & Hey, 2015). B-vitamins found in wheat grain are Niacin, Riboflavin, Thiamin, Folate and B6 (Piironen & Salmenkallio-Marttila, 2009). Therefore, these nutritional components are prevalent in whole wheat foods but lacking in refined wheat flour. Breeding for high yield has been the major focus in the past, but providing sufficient minerals is an important target, too (Poole *et al.*, 2021). Wheat supplies minerals and micronutrients, but especially Iron (Fe), Zinc (Zn), Copper and Manganese contents are insufficient (Piironen & Salmenkallio-Marttila, 2009). Taking into account that bioavailability of micronutrients is also hampered by anti-nutritive compounds such as phytate, wheat might not be able to provide adequate supply of micronutrients. Therefore, especially in regions where wheat is a major staple, it needs to provide a significant share of macro- and micronutrients for a healthy diet. Yet grain quality is a highly variable trait, depending on genotype, cultivation and especially environmental effects. Therefore, improvement of grain quality can be achieved by various approaches, such as breeding with exotic germplasm (Monasterio & Graham, 2000; Cakmak *et al.*, 2004; Chhuneja *et al.*, 2006; Johns & Eyzaguirre, 2007; Rawat *et al.*, 2009; Chatzav *et al.*, 2010), biotechnology approaches (Shewry, 2007; Shewry, 2009; Ali & Borrill, 2020) and agronomic practices for biofortification (Johns & Eyzaguirre, 2007; Cakmak *et al.*, 2010).

1.4 Aims and Objectives

Wheat plays a pivotal role in food security and providing nutritious food. Wild wheats harbor beneficial grain quality traits that were altered during domestication and subsequent breeding in modern wheat cultivars (Cakmak *et al.*, 2000; Arora *et al.*, 2019; Biel *et al.*, 2021; Debnath *et al.*, 2023). Unique wild traits, e.g. nutritional quality, could be retained via a *de novo* domestication that facilitates the use of CWRs directly by only modifying selected genes for cultivation (Zsögön *et al.*, 2018). Therefore, a *de novo* domestication of wild wheat would bypass the effect of domestication and maintain favorable nutritional traits. The aim of this thesis was to explore the wild *Triticum* taxa for their agricultural and domestication potential and thus to pave a way for a *de novo* domestication of wild wheat with beneficial grain quality.

An overview of *Triticum*'s evolutionary history, origin and wild taxa in the *Triticum* genepool was provided (Chapter 2). Further, grain quality traits of wheat relatives and their evolutionary pattern were reviewed. The current knowledge on grain Fe and Zn concentration including a quantitative meta-analysis in wheat relatives, their potential for improved baking quality by glutenin subunits and celiac disease safe gliadins and their antioxidant properties was summarized. Based on this review, knowledge gaps on neglected wild wheat taxa and on the *Triticum* taxa regarding grain quality traits were identified (Chapter 2, Zeibig *et al.* (2022)).

For a *de novo* domestication, the wild wheats need to be able to grow in the selected environment. Wheat's origin is located in the Fertile Crescent (Feldman & Levy, 2023) and therefore the climatic conditions of the central European climate that was chosen as a target environment differ (Jaradat, 2017). The suitability of the temperate central European climate as a target environment for the wild *Triticum* taxa, including neglected taxa identified in Chapter 2, was assessed (Chapter 3, Zeibig *et al.* (2024a)). Furthermore, domestication changed traits and underlying genetic base of crops that enabled their cultivation. The phenotypic differences between wild and domesticated and modern wheat cultivars were analyzed and derived target traits and genes that should be priority for a *de novo* domestication were proposed (Zeibig *et al.*, 2024a).

Domestication not only altered the phenotype of wheat but also the grain quality and its composition. The literature review in Chapter 2 provided insights on beneficial grain micronutrients Fe and Zn concentrations in wild wheat compared to modern cultivars. The accumulation of high grain Fe and Zn concentration was associated with higher Grain Protein Content (GPC). For the Total Phenolic Content (TPC), no clear pattern was identified (Zeibig *et al.*, 2022). To elucidate the alteration of these wheat grain quality traits by domestication, the differences in grain quality between the wild *Triticum* wheats and their domesticated landrace taxa that showed a good suitability to the temperate central European climate were analyzed (Chapter 4, Zeibig *et al.* (2024b)). The GGAA wheats were recognized as neglected taxa regarding grain quality analyses and therefore the untapped grain quality potential of the GGAA wheats was identified (Chapter 4). Based on their grain quality characteristics, selected genotypes for a *de novo* domestication project were proposed. The results of this project will help to elucidate the scope of using wild wheats via *de novo* domestication to develop diversified and balanced diets and offering an alternative pathway for wheat breeding.

2 The grain quality of wheat wild relatives in the evolutionary context

Frederike Zeibig[1], Benjamin Kilian[2], Michael Frei[1]

[1] – Department of Agronomy and Crop Physiology, Institute of Agronomy and Plant Breeding I, Justus-Liebig-University, 35392, Giessen, Germany

[2] – Global Crop Diversity Trust, 53113, Bonn, Germany

Published in: Theoretical and Applied Genetics, 135, 4029–4048 (2022)

DOI: 10.1007/s00122-021-04013-8

Theoretical and Applied Genetics (2022) 135:4029–4048
https://doi.org/10.1007/s00122-021-04013-8

REVIEW

The grain quality of wheat wild relatives in the evolutionary context

Frederike Zeibig[1] · Benjamin Kilian[2] · Michael Frei[1]

Received: 27 July 2021 / Accepted: 6 December 2021 / Published online: 17 December 2021

Abstract

Key message **We evaluated the potential of wheat wild relatives for the improvement in grain quality characteristics including micronutrients (Fe, Zn) and gluten and identified diploid wheats and the timopheevii lineage as the most promising resources.**

Abstract Domestication enabled the advancement of civilization through modification of plants according to human requirements. Continuous selection and cultivation of domesticated plants induced genetic bottlenecks. However, ancient diversity has been conserved in crop wild relatives. Wheat (*Triticum aestivum* L.; *Triticum durum* Desf.) is one of the most important staple foods and was among the first domesticated crop species. Its evolutionary diversity includes diploid, tetraploid and hexaploid species from the *Triticum* and *Aegilops* taxa and different genomes, generating an AA, BBAA/GGAA and BBAADD/GGAAA^{m}A^m genepool, respectively. Breeding and improvement in wheat altered its grain quality. In this review, we identified evolutionary patterns and the potential of wheat wild relatives for quality improvement regarding the micronutrients Iron (Fe) and Zinc (Zn), the gluten storage proteins α-gliadins and high molecular weight glutenin subunits (HMW-GS), and the secondary metabolite phenolics. Generally, the timopheevii lineage has been neglected to date regarding grain quality studies. Thus, the timopheevii lineage should be subject to grain quality research to explore the full diversity of the wheat gene pool.

Introduction

Wheat is one of the world's most important crops. It provides 19% of human calorie intake and 21% of protein intake (Shiferaw et al. 2013). Hexaploid bread wheat (*Triticum aestivum* L., $2n = 6x = 42$, BBA^{u}A^{u}DD) accounts for about 90% of wheat production, and tetraploid pasta wheat (*Triticum durum* Desf., $2n = 4x = 28$, BBA^{u}A^u) is the 10th most important staple crop (International Grains Council [IGC] (2020). World Grain Statistics 2016). The two economically most important wheat species have different levels of ploidy. This is due to the evolutionary history, in which the process of domestication plays a predominant role (Dubcovsky and Dvorak 2007). Domesticated plants fulfilled the requirements of human cultivation and dietary preference. The main characteristic of domesticated cereals is the loss of the ability to survive independently in the wild (Meyer and Purugganan 2013; Purugganan and Fuller 2009; Zohary et al. 2012). The center of wheat diversity and initial domestication lies in the Fertile Crescent. The evolutionary history of wheat is characterized by the allo-polyploidization of the originally diploid *Triticum* and *Aegilops* species, which led to the emergence of tetra- and hexaploid wheat species. Human cultivation of the tetraploid wild emmer *Triticum dicoccoides* (Körn. ex. Aschers. & Graebner) Schweinf. ($2n = 4x = 28$, BBA^{u}A^u) resulted in the emergence of the domesticated *Triticum dicoccon* Schübl. ($2n = 4x = 28$, BBA^{u}A^u) and later *T. durum*. The domesticated tetraploid species as well as *T. aestivum*, of which no direct hexaploid wild relative has yet been identified, expanded their cultivation area by human migration and trade (Kilian et al. 2010; Zohary et al. 2012). Migration from their center of diversity and continuous breeding efforts reduced the genetic diversity of *T. aestivum* and *T. durum* (Tanksley and McCouch 1997; Ross-Ibarra et al. 2007). Currently, climatic conditions are changing, posing a threat to the productivity of the cultivated wheats (Zhao et al. 2017; Iizumi et al. 2021; Trnka et al. 2014). The wild and domesticated relatives of wheat are a valuable source of beneficial genetic diversity

---

✉ Michael Frei
michael.frei@agrar.uni-giessen.de

[1] Department of Agronomy and Crop Physiology, Institute of Agronomy and Plant Breeding I, Justus-Liebig-University, 35392 Giessen, Germany

[2] Global Crop Diversity Trust, 53113 Bonn, Germany

that can be used for crop improvement (Dempewolf et al. 2014; Henry and Nevo 2014; Brozynska et al. 2016; Kilian et al. 2021; Sharma et al. 2021). Wild relatives of wheat are well adapted to their natural habitats, which can be harsh, so they carry useful traits for abiotic stress tolerance (Henry and Nevo 2014; Singh et al. 2012). Their genetic diversity has also been exploited for their resistance to various pests, e.g., hessian fly (Nsarellah et al. 2003) and diseases, such as powdery mildew (Li et al. 2020a) and leaf rust (Fatima et al. 2020; Narang et al. 2020). These beneficial traits can be incorporated into modern wheat cultivars via (pre-)breeding programs (Kilian et al. 2011, 2021; Sharma et al. 2021) and thus illustrate the value of this genepool for securing the future cultivation of wheat. In order to harness and conserve the beneficial diversity of wild relatives, genebanks play an important role as ex situ conversation sites (Dempewolf et al. 2014; Kilian et al. 2021). An overview of ex situ *Triticum* collections can be found in Sharma et al. 2021.

Due to wheat's role as a staple, the quality of the grain is of great importance. Its daily consumption in many parts of the world makes it a major source for calorie intake, but it also provides micronutrients and vitamins. Breeding for energy yield and grain quality for purposes such as baking has long been a major goal in wheat breeding and enabled food security for a growing population. However, the focus on micronutrients and allergenicity has only been a recent trend in wheat breeding. Especially in less diversified diets, a low concentration of micronutrients in wheat appears to be a major problem. This leads to micronutrient deficiencies ("hidden hunger"), especially for the crucial micronutrients iron (Fe) and zinc (Zn) (Graham et al. 2001, 2007; Johns and Eyzaguirre 2007). The main symptom of Fe deficiency is anemia, and Zn deficiency can affect growth and development (Prasad et al. 1961; Stein 2010; Gibson et al. 2010). To combat hidden hunger, one commonly used approach is biofortification (=enrichment of micronutrients) of crops (Johns and Eyzaguirre 2007). Crop biofortification can be achieved through agronomic strategies such as foliar fertilization or via various breeding methods such as introgression. Some wild relatives of wheat accumulate micronutrients very efficiently in the grain and offer high genetic diversity for this trait (Arora et al. 2019; Cakmak et al. 2000; Chhuneja et al. 2006; Chatzav et al. 2010). Therefore, they can be used as a breeding resource for biofortified crops (Cakmak et al. 2004; Rawat et al. 2009).

Wheat is consumed in various forms, for example as bread, pasta, bulgur and couscous. An important quality-determining characteristic is gluten, which comprises a group of seed storage proteins composed of glutenins and gliadins. While the former is very important for dough quality and elasticity, the latter is responsible for viscosity (Wieser 2007; Shewry 2019). However, gliadins can trigger an autoimmune disease called celiac disease (CD) (Biesiekierski 2017). Currently, the only strategy for CD patients is to avoid wheat products. The wheat genepool may harbor diversity that can help to combine improved dough quality properties of glutenin with less toxic and non-immunogenic gliadin variants for CD-safe wheat.

Phenolics are bioactive components in the grain with promising health benefits for the consumer (Laddomada et al. 2015). Phenolic compounds can be grouped into phenolic acids, flavonoids, stilbenes, coumarines, lignans and tannins. Phenolic acids are the most abundant component in wheat and are predominantly stored in the aleurone layer and the bran (Liyana-Pathirana and Shahidi 2006; Laddomada et al. 2015). The domesticated einkorn and emmer were assumed to be enriched in phenolic acid content (Serpen et al. 2008; Barański et al. 2020), and thus, wild wheats could be a valuable source for the enrichment of phenolic acids in the whole wheat grain.

This review provides an overview of the state of research on grain quality traits in the wheat genepool, including micronutrients, gluten and phenolics. Research on grain quality parameters in wheat relatives is summarized and assessed in the context of evolutionary history.

The process of domestication

Humans selected wild plants with favorable characteristics for cultivation and preparation to ensure food security. This process genetically transformed the plants into a domesticated plant that is dependent on human care and thus loses its ability to survive in the wild (Doebley et al. 2006). The modified plants showed a distinctive morphology that is referred to as the domestication syndrome (Hammer 1984). The domestication syndrome evolved from the selection of wild plants for higher germination rates and easier harvesting (Purugganan and Fuller 2009). The most common traits are the loss of seed shattering, increased seed size and alteration of the reproductive organs (Doebley et al. 2006; Ross-Ibarra et al. 2007; Meyer and Purugganan 2013). These domestication traits evolved through a combination of conscious and unconscious selection. Humans actively carried out conscious selection by selecting a favored trait and thus forwarded this trait into the next generation. Unconscious selection, on the other hand, favored the adaptability to the new agro-ecological environment shaped by human farming systems. As a result, the plant improved its performance in the field and was chosen for further breeding (Zohary 2004). In the beginning, wild and cultivated plants grew close to each other, which led to the exchange of genes via cross-pollination and gene flow (Gross and Olsen 2010; Przewieslik-Allen et al. 2021; He et al. 2019). This scenario still occurs today in the centers of origin of crops. However, human intervention has morphologically and genetically modified the wild plants, resulting in a newly domesticated species (Kantar et al. 2017). In addition to selection for domestication traits, selection for plant improvement further differentiated the domesticated species. This process is called diversification phase and involves the adaption of plants to new environments and selection for quality traits that differed individually for each crop (Meyer and Purugganan 2013). As a result, the differently pronounced selection pressure led to further divergence within the species. Genetic alteration and introduction into new habitats led to adaptation of a domesticated species. In summary, the domestication and improvement phase increased diversity in terms of the number of newly emerged domesticated taxa. However, it is important to focus on the initial situation of domestication. The onset of domestication occurred independently in different areas around the world at different times, but the traits of interest were similar (Purugganan and Fuller 2009; Meyer and Purugganan 2013). From the broad wild diversity that was available to humans, only a few plants were selected and used as founder crops (Ladizinsky 1985). Consistent cultivation and selection within these founder crops led to a genetic bottleneck that excluded many wild alleles from the domestication process (Doebley et al. 2006; Stetter et al. 2017). The diversity retrieved during the domestication and improvement phase cannot compensate for the reduced diversity at the beginning (Smýkal et al. 2018). The founder crop (population) experienced high selection pressure on domestication traits. Increased selection led to selective sweeps that are reflected in a reduced diversity of domestication loci compared to other loci (Smýkal et al. 2018; Maccaferri et al. 2019; Pont et al. 2019). Most early domesticates were annuals because the fixation of traits through an annual selection cycle was more successful and faster compared to perennials. Thus, selection pressure on desired traits accelerated the loss of diversity in the corresponding loci and reduced overall diversity (Maccaferri et al. 2019).

Domestication history of wheat and its resulting diversity

Wheat's allo-polyploidy accelerated its wider distribution, as polyploid species show a better adaption rate to new environments and outperform their ancestors with a lower degree of ploidy in agronomic traits (Dubcovsky and Dvorak 2007). Therefore, farmers favored them, which led to a larger cultivation area and facilitated their spread from the Fertile Crescent across the world.

Diploid wheats

Diploid wheats (AA) belong to the oldest cultivated plant species. Wild representatives of the diploid wheat group are *Triticum boeoticum* Boiss ($2n=2x=14$, AA) and *Triticum urartu* Thumanjan ex Gandilyan ($2n=2x=14$, A^uA^u). *Triticum monococcum* L. ($2n=2x=14$, AA) is the domesticate of *T. boeoticum*. Together, they form the einkorn group of wheat. The Karacadağ and Kartal-Karadağ mountains in Turkey have been identified as their center of domestication (Heun 1997; Kilian et al. 2007b). From here, einkorn migrated east (Armenia, Georgia, Iran) and west via the Bosporus (Greece and toward Central Europe). Another route involved maritime transport to the Maghreb and the Iberian Peninsula. These routes are supported by archaeological evidence, but also the population structure of the einkorn genepool that was shaped by the migration routes (reviewed in: Zaharieva and Monneveux 2014; Brandolini et al. 2016) (Fig. 1).

T. urartu is phenotypically similar to *T. boeoticum* (Morrison 1993) but can be distinguished genetically (Yildirim and Akkaya 2006; Konovalov et al. 2010). *T. urartu* represents the A genome donor of tetra- and hexaploid wheats (Huang et al. 2002b; Yildirim and Akkaya 2006). *T. urartu* populations occur in the Fertile Crescent and are found in Lebanon, Turkey, Iraq, Iran and Transcaucasia. This geographic distribution also shaped the genetic diversity of *T. urartu* by forming groups according to bioclimatic and topographic criteria (Brunazzi et al. 2018).

Tetraploid wheats

Hybridization between *T. urartu* and a close relative of *Aegilops speltoides* Tausch ($2n=2x=14$, BB/GG), a member of the *Sitopsis* section in *Aegilops*, initiated the formation of the tetraploid wild wheat, *T. dicoccoides*. This wild emmer wheat further evolved into the domesticated *T. dicoccon*. However, the emmer lineage is not the only tetraploid wild wheat. *Triticum araraticum* Jakubz. ($2n=4x=28$, $GG{}^uA^u$) is also a descendant of an independent hybridization between *T. urartu* and *Ae. speltoides*. Although both tetraploid wheat lineages share potentially the same parental species, they differ in the structure of the genome and time of origin (Adonina et al. 2015). Diversity analysis clearly showed a shared ancestry of *Ae. speltoides* and tetra- and hexaploid wheats. *Ae. speltoides* shared nuclear and cytoplasmic loci with *T. dicoccoides* and *T. araraticum*, which differed between the two wild tetraploid wheat species. Therefore, it was proposed that both genomes were derived (at least in parts) from *Ae. speltoides* as a maternal donor (Kilian et al. 2007a; Golovnina et al. 2007). However, the G genome of *T. araraticum* shows a closer relationship with *Ae. speltoides*, while the B genome donor is more distant to *Ae. speltoides* (Huang et al. 2002b; El Baidouri et al. 2017; Bernhardt et al. 2017).

Fig. 1 Origin and spread of domesticated einkorn wheat *T. monococcum*. Green dashed fields represent the Fertile Crescent; red circle is the domestication region of *T. monococcum* (Heun 1997; Kilian et al. 2007b); arrows indicate T. *monococcum*'s dispersal pathways (Zaharieva and Monneveux 2014; Brandolini et al. 2016)

Domestication and cultivation of the BBAA lineage and the hexaploid BBAADD

Molecular analysis of *T. dicoccoides* revealed two subgroups with distinct geographic origin. The southern Levant subgroup included genotypes from Israel, Palestine, Jordan, Syria and Lebanon. Accessions belonging to the other group were associated with the Central Eastern Fertile Crescent extending from Turkey to Iraq and Iran (Luo et al. 2007; Ozkan et al. 2005; Maccaferri et al. 2019). Some studies suggested a monophyletic origin of domesticated emmer from within the Central Eastern group. However, archaeological data indicate early cultivation of emmer in the Southern Levant (reviewed in: Özkan et al. 2011). Recent analysis suggested a multi-regional origin by showing that domesticated emmer contains alleles from both *T. dicoccoides* populations. It was concluded that wild emmer was pre-cultivated in the southern Levant and met wild emmer from the Central Eastern group through migration northwards, where it was eventually domesticated (Oliveira et al. 2020). *T. dicoccon* spread in all directions via various routes. Europe was reached via the Bosporus, the Balkans and the Iberian Peninsula. Africa was reached via the southern Levant and the Arabian Peninsula, and the spread to Asia was via Iran (reviewed in: Martínez-Moreno et al. 2020; Badaeva et al. 2015) (Fig. 2).

T. durum is currently the economically most important cultivated species from the tetraploid BBAA genepool. Durum wheat most likely originated in the eastern Mediterranean region (Luo et al. 2007; Kabbaj et al. 2017), which is supported by a high genetic diversity in durum wheat accessions from the Central Fertile Crescent (Baloch et al. 2017). However, its evolution has not yet been fully elucidated. Phylogenetic analysis of wild and domesticated emmer, durum wheat landraces and durum cultivars revealed a close relationship between durum wheat landraces and domesticated emmer from the southern Levant. This suggests a rather linear evolution, in which increased selection and improvement on *T. dicoccon* facilitated the emergence of *T. durum* landraces. Moreover, modern durum wheat cultivars showed the closest relationship to durum wheat landraces from North Africa and Transcaucasia (Maccaferri et al. 2019). In contrast, exome sequencing data of the wheat genepool identified *T. dicoccoides* as a direct donor to *T. dicoccon* and *T. durum*, suggesting an entangled evolution (Pont et al. 2019). From its potential center of origin, durum wheat spread widely following similar expansion routes as *T. dicoccon* (Moragues et al. 2007; Kabbaj et al. 2017; Martínez-Moreno et al. 2020) (Fig. 2).

The emmer lineage evolved further via another hybridization event, which gave rise to the hexaploid wheats. Most likely, domesticated tetraploid wheat and *Aegilops tauschii* Cosson ($2n = 2x = 14$, DD) hybridized in the area of northeastern Iran and the southwestern Caspian Sea, giving rise to the hexaploid bread wheat, *T. aestivum* (Petersen et al. 2006; Salamini et al. 2002; Huang et al. 2002a; Pont et al. 2019). The highest genetic diversity for hexaploid bread wheat was found in the Near and Middle East, which is probably the center of diversity (Huang et al. 2002a). Hexaploid bread wheat is based on a founder race that further evolved into

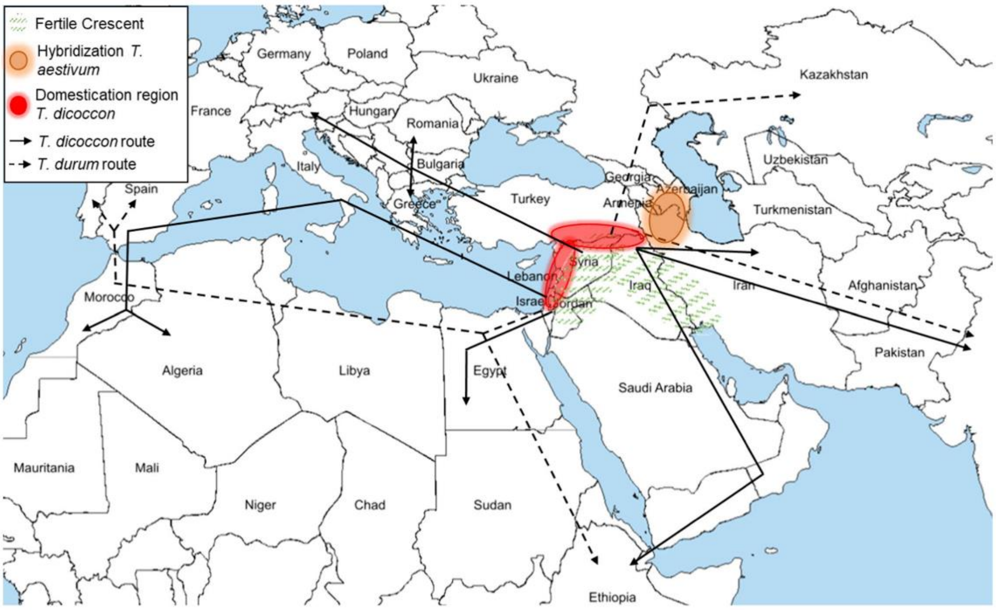

Fig. 2 Origin and spread of domesticated emmer and durum wheat. Green dashed fields are the Fertile Crescent; red circles are the domestication regions of *T. dicoccon* (Oliveira et al. 2020); solid arrows indicate dispersal routes of *T. dicoccon* (Martínez-Moreno et al. 2020; Badaeva et al. 2015); dashed arrows indicate dispersal route of *T. durum* (Moragues et al. 2007; Kabbaj et al. 2017; Martínez-Moreno et al. 2020); orange circle is the potential hybridization site of *T. aestivum* (Petersen et al. 2006; Salamini et al. 2002; Huang et al. 2002a; Pont et al. 2019)

two hexaploid wheat genepools (Pont et al. 2019). Moreover, the wheat population can be divided into a western and far eastern subpopulation, the latter resembling the ancient wheat due to less introgression from *Ae. tauschii* (Wang et al. 2013). Polyploidization limited the initial diversity of the new species, as only a small number of genotypes were potentially involved in its emergence (Dubcovsky and Dvorak 2007; Pont et al. 2019). However, due to introgression from *T. dicoccoides* into the hexaploid wheat genome, some diversity was retained (He et al. 2019).

Domestication and cultivation of the wheat G genome containing lineage

In contrast to the emmer lineage, the timopheevii lineage with the genomic constitution GGAA received less attention. The evolutionary history of the GGAA wheat genepool is complex, as several chromosomal rearrangements were involved (Badaeva et al. 2021). The wild ancestral form of the timopheevii lineage is *T. araraticum*. Northern Iraq is the center of diversity and origin of *T. araraticum* (Gornicki et al. 2014; Badaeva et al. 2021) (Fig. 3). *T. araraticum* comprises two subgroups. One subgroup (ARA-0) is widespread, while the other (ARA-1) was only found in south-eastern Turkey and north-western Syria (Badaeva et al. 2021).

Triticum timopheevii (Zhuk.) Zhuk ($2x = 4n = 28$, GGA^uA^u) is the domesticate of *T. araraticum*. The origin of *T. timopheevii s.str.* (found in Georgia) remains unclear, but was probably introduced from Turkey (Badaeva et al. 2021). Badaeva et al. (2021) also discuss the potential sister-group relationship between the Georgian *T. timopheevii s.str.* and the prehistoric and widespread *T. timopheevii s.l.* ('New Glume Wheat'), with the oldest known records being of Turkish origin.

The mixed cultivation of *T. monococcum* and *T. timopheevii* in western Georgia facilitated hybridization between the two. This event resulted in the hexaploid *Triticum zhukovskyi* Men. & Er. ($2n = 6x = 42$, $GGA^uA^uA^mA^m$) (Badaeva et al., 1994; Badaeva et al., 2016; Badaeva et al., 2021) (Fig. 3). However, Pont et al. (2019) suggested a hybridization between *T. araraticum* and *T. boeoticum* for the formation of *T. zhukovskyi*.

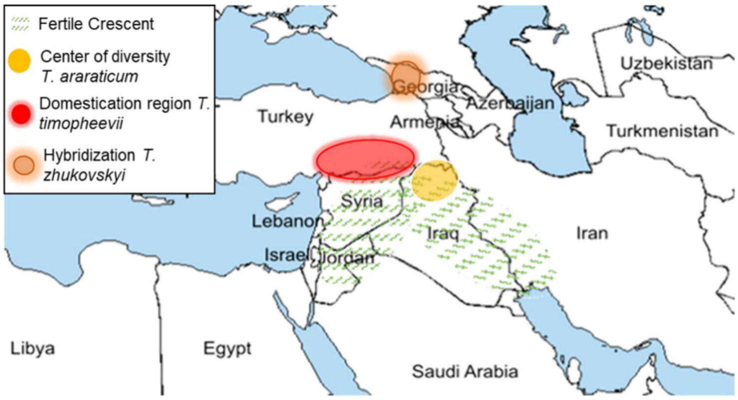

Fig. 3 Origin and spread of GGAA wheat. Green dashed fields are the Fertile Crescent; yellow circle indicates the center of diversity of *T. araraticum* (Gornicki et al. 2014; Badaeva et al. 2021); red circle is the domestication region of *T. timopheevii* (Mori et al. 2009; Badaeva et al. 2021); orange circle indicates the hybridization site of *T. zhukovskyi* (Badaeva et al. 1994; Badaeva et al. 2016; Badaeva et al. 2021)

Micronutrients in wheat wild relatives

Iron (Fe) and Zinc (Zn) deficiency are major forms of micronutrient disorders in human diets relying on one major crop. Fe deficiency leads to anemia and insufficient Zn uptake impairs various essential functions of the body including growth, development and the immune system (Stein 2010). The large wheat genepool described earlier may harbor beneficial genetic variation for an increased accumulation of micronutrients in the edible parts of the wheat crop. Furthermore, the evolutionary pattern can be used to identify trends and underutilized species for biofortification.

Wheat wild relatives show high enrichment and variation for Fe and Zn in the grain

Aegilops species and the einkorn and emmer lineage were of interest for the grain micronutrient concentration and the variation. The wild species harbored a high variation for Zn grain concentration, whereas *Aegilops* species showed elevated Fe grain concentration.

Domesticated einkorn wheat forms a valuable source for biofortification, especially for Zn (Table 1) (Ozkan et al. 2007; Erba et al. 2011). In addition, high values of Fe and Zn were consistently found in *T. monococcum* accessions across different locations (Ozkan et al. 2007; Erba et al. 2011). Therefore, an underlying genetic component is likely. Compared to *T. monococcum*, the variation in Zn and Fe concentrations was higher in *T. boeoticum* in the studies comparing the wild and domesticated forms (Fig. 4, Table 1). This underlines the greater potential for genetic variation in crop wild relatives. Furthermore, grains of *T. zhukovskyi* could be potentially enriched in Zn, due to the fact that it contains the A genome of *T. monococcum*. Among the few studies available, most focused on *T. monococcum* and *T. boeoticum* and excluded *T. urartu*, the actual A genome donor of emmer, durum, bread wheat and timopheevii wheat. Because of this ancestry, exploring the micronutrient content and accumulation of *T. urartu* might be valuable for the biofortification of its descendants. Diploid wheats have been less extensively investigated than their tetraploid relatives from the emmer lineage. One reason for this lack of interest could be their inferior agricultural performance or the challenges in introgressing beneficial alleles into bread wheat. Thus, there is still a research gap that needs to be explored.

The same is true for *Aegilops* accessions, which have been investigated only sporadically, although some accessions could be a promising resource for biofortification. Compared to cultivated bread wheat, *Ae. tauschii* and *Ae. kotschyi* showed higher grain Fe and Zn concentrations (Chhuneja et al. 2006; Rawat et al. 2009) (Table 1).

Tetraploid wild emmer wheat (*T. dicoccoides)* has always received attention as this species provides a valuable source of genetic variation in terms of accumulation of micronutrients (Table 1) (Cakmak et al. 2000; Peleg et al. 2008). Higher accumulation of Fe and Zn in *T. dicoccoides* compared to *T. durum* was also consistent under water deficient conditions (Peleg et al. 2008). Compared to modern wheat cultivars, wild emmer wheat showed a higher variation in Zn and Fe concentration (Cakmak et al. 2004). Comparing the variation between Fe and Zn in wild diploid *T. boeoticum* and wild tetraploid emmer wheat, the amplitude for Zn was higher than for Fe (Cakmak et al. 2000).

Studies that analyzed iron and/or zinc in wild wheat accessions were summarized to calculate an overall mean (mg/kg) (Table 1). Further, the mean value of species analyzed in at least two independent studies was used for comparison between species. Due to the small number of studies, the median and the individual values were visualized (Fig. 4) (Weissgerber et al. 2015). *Aegilops* accessions showed higher iron accumulation compared to *Triticum* taxa across different studies (Fig. 4). However, the highest value

Table 1 Summary of studies in which more than one genotype of the respective species was examined for Fe and Zn concentration in the grain

Species	Fe					Zn				
	Mean (mg/kg)	Range (mg/kg)	Difference (mg/kg)	Number of Studies	References	Mean (mg/kg)	Range (mg/kg)	Difference (mg/kg)	Number of Studies	References
Ae. cylindrica	67	52–93	41	1	5	39	32–52	20	1	5
Ae. geniculata	70	52–82	30	1	5	38	32–41	9	1	5
Ae. kotschyi	74	23–91	68	2	4,5	59	22–59	37	2	4,5
Ae. longissima	73	60–82	22	1	5	42	25–51	26	1	5
Ae. peregrina	53	34–82	48	1	5	40	33–49	16	1	5
Ae. tauschii	71	30–99	69	3	2,4,9	48	18–69	51	3	2,4,9
Ae. ventricosa	66	55–94	39	1	5	34	24–39	15	1	5
T. aestivum	35	21–51	30	5	1,4,5,7,8	26	15–61	46	5	1,4,5,7,8
T. araraticum	30	23–59	36	1	5	24	19–31	12	1	5
T. boeoticum	51	24–92	68	3	1,2,5	54	22–177	155	3	1,2,5
T. dicoccoides	43	15–109	94	3	1,3,5	64	14–190	176	4	1,2,3,5
T. dicoccon	34	31–40	9	1	8	43	16–135	119	2	2,8
T. durum	31	10–50	40	3	1,5,8	23	14–50	36	3	1,5,8
T. monococcum	48	32–85	53	4	1,6,7,8	52	28–89	61	4	1,6,7,8

References: (1) Cakmak et al. 2000; (2) Monasterio and Graham, 2000; (3) Cakmak et al.2004; (4) Chhuneja et al.2006; (5) Rawat et al. 2009; (6) Ozkan et al. 2007; (7) Erba et al. 2011; (8) Zhao et al. 2009; (9) Arora et al. 2019

Fig. 4 Distribution of mean Fe or Zn grain concentration measured in different studies for different species. The bold lines are the median of different species means. Dots represent individual mean values. Excel script from Weissgerber et al. (2015)

for grain Fe concentration in *T. boeoticum* was in the same range as the median Fe concentration in *Ae. kotschyi* and *Ae. tauschii*. Such clear differentiation was not present for grain Zn concentration across species. The variation in grain Zn concentration was higher, as shown by the wider distribution of the mean values. This variation was pronounced in *T. dicoccoides*, which showed the highest median for grain Zn concentration (Fig. 4). This confirms the observations that the variation is higher for Zn accumulation than for Fe. It is noteworthy that this enrichment and variation found in wild emmer has not been passed on and confirmed in its economically important progeny *T. durum* (Fig. 4).

Factors influencing the Fe and Zn concentration in the grain

How, if and to what extend the micronutrient concentration in the grain is influenced by external factors and the phenotype, e.g., grain size, is contradictory to date. Some authors argue that enhanced micronutrient density may come at the expense of agronomic performance. Progress in breeding for higher yields may have diluted the concentration of micronutrients in the grain, which is referred to as the dilution effect or reversible concentration effect in small grains (Cakmak et al. 2000). This hypothesis is supported by observations of negative correlations between yield and grain Zn concentrations (Oury et al. 2006). Negative correlation also occurred between the year of release of the respective variety and its micronutrient density (Zhao et al. 2009), suggesting that the breeding for high yield negatively affects micronutrient concentrations. This observation was more pronounced for Zn compared to Fe (Oury et al. 2006; Morgounov et al. 2007). On the other hand, some studies found no correlation between seed size and micronutrient concentration, calling into question the hypothesis of the dilution effect (Arora et al. 2019; Distelfeld et al. 2007). The micronutrient content per seed was analyzed in addition to the concentration. Seeds with the highest concentration also had the highest content per seed due to high seed weight and size (Cakmak et al. 2004, 2000). This clearly refutes the dilution hypothesis. In conclusion, the results concerning the relationship between agronomic performance and micronutrient density are controversial, suggesting that both components contribute to micronutrient density.

To identify the traits associated with grain micronutrient concentrations, correlations with other grain characteristics were analyzed. Agronomic management practices can help to increase micronutrient concentrations in the grain. Plants grown under nitrogen-sufficient conditions showed enriched micronutrients levels compared to nitrogen-deficient conditions (Monasterio and Graham 2000). Furthermore, higher grain protein content was also associated with higher micronutrients (Chatzav et al. 2010; Peleg et al. 2008). Fe and Zn are preferentially stored in the aleurone layer and the embryo. At the same time, the embryo contains high protein contents, which can explain the positive associations (Morgounov et al. 2007). As nutrients are translocated from the leaves to the grain during grain filling, nutrient concentrations (N, Fe, Zn) in the flag leaf were correlated with grain concentrations (Rawat et al. 2009). These findings suggest that grain protein is a sink for micronutrients and explain why bread wheat shows lower micronutrient concentration than durum wheat (Cakmak et al. 2010). In conclusion, an adequate supply of nitrogen can help to exploit the full potential of micronutrient accumulation in the grain. However, there is also another genetic component that supports wild wheats in the enrichment of micronutrients in the grain.

Genetic properties of micronutrient accumulation in wheat wild relatives

Micronutrient uptake and translocation are quantitatively controlled and inherited traits (Arora et al. 2019) that can be genetically dissected by genome mapping. Therefore, Recombinant Inbred Lines (RIL) from two domesticated einkorn lines were developed by Ozkan et al. (2007) in order to gain insights into the genetics of micronutrient uptake. They found a promising Quantitative Trait Loci (QTL) on the short arm of chromosome 5 that contributes micronutrient accumulation (Ozkan et al. 2007). Chromosome 5A also harbored favorable QTLs for Fe and Zn micronutrient concentrations in a tetraploid RIL population derived from durum wheat and wild emmer. The authors stated that the wild alleles increased micronutrient accumulation (Peleg et al. 2009). Furthermore, chromosome 6B was detected as a promising gene source conferring high micronutrient concentrations in grains (Peleg et al. 2009; Cakmak et al. 2000, 2004). RIL lines containing chromosome 6B of *T. dicoccoides* had an enriched Zn concentration in the grain (Cakmak et al. 2000, 2004). Independent of this finding, the QTL *GPC-B1* on the short arm of chromosome 6B of *T. dicoccoides* was associated with elevated grain protein concentration (Joppa et al. 1997). *GPC-B1* showed a direct influence on chlorophyll degradation of flag leaves, thus accelerating senescence, and had pleiotropic effects on grain protein (Uauy et al. 2006a). Consequently, the transportation mechanism of nitrogen from leaves to grains was investigated for its role in grain micronutrient content. Recombinant chromosome substitution lines carrying the *GPC-B1* allele from wild emmer wheat showed increased Zn and Fe concentration in the grain compared to the *GPC-B1* allele from durum wheat (Distelfeld et al. 2007). The QTL was narrowed down to the allele *NAM-B1*, which encodes a NAC transcription factor (Uauy et al. 2006b). Reduced expression of *NAM-B1* resulted in plants with lower levels of protein, Zn and Fe in the grain, but higher levels in the flag leaf. This proved the role of *NAM-B1* in remobilization of micronutrients and nitrogen (Uauy et al. 2006b) and further explained the observed positive correlation between these components described above. Only wild wheat species possessed the functional *NAM-B1* genes (*GPC-B1* gene, used synonymously), leading to higher accumulation of micronutrients compared to *T. durum* and *T. aestivum*, where the allele is non-functional. Hexaploid wheat contains two functional homologous genes on chromosomes 6A and 6D (Uauy et al. 2006b). This genetic alteration occurred during wheat evolution, emphasizing the benefit of wild relatives. Therefore, the genomes of wheat relatives were explored for similar loci. Orthologues of *NAM-B1* were identified in *T. timopheevii*, *Ae. speltoides*, *Ae. bicornis*, *Ae. longissima*, *Ae. searsii*, and *Ae. sharonensis* (Hu 2012; Hu et al. 2013). Apart from some Single Nucleotide Polymorphisms (SNP), the *T. timopheevii NAM-G1* orthologue resembled the *T. dicoccoides NAM-B1* gene in promoting grain protein content and was therefore considered as a putative target for micronutrient enrichment. However, the follow-up study by Hu et al. (2017) showed no association between *NAM-G1* expression levels and grain Fe and Zn concentration. Consequently, they proposed a quantitatively controlled trait for micronutrient concentrations in grain rather than just *NAM-G1* as a single gene causing the higher micronutrient content (Hu et al. 2017). Another explanation for this finding could be that the allele does not function properly due to *T. timopheevii*'s domestication status. To confirm this, a study with its wild ancestor *T. araraticum* would be required. Additionally, *Ae. speltoides* or an extinct close relative of it participated as donors for the B and G genome. However, different genoytpes were involved in the independent polyploidization events of the emmer and timopheevii lineage. Since the functional *NAM* gene is found on the B1 genome, it might be non-functional in G1, thus explaining the lower micronutrient concentration in *T. timopheevii*.

Regulatory role of the *GPC* gene

The physiological implications of this wild gene in hexaploid wheats were studied to elucidate its role in metal homeostasis. The pre- and post-anthesis phases are the most critical for micronutrient accumulation. Fe and Zn are not directly transferred into the grain. Initially, they are stored in the leaves, where they play an important role in the function of essential enzymes in mitochondria and plastids (Gupta et al. 2020). *GPC* is expressed during early senescence to prepare the plant for subsequent physiological processes (Cantu et al. 2011). Transcriptome analysis showed that under a high *GPC* expression levels, genes involved in transport, protein metabolism and catabolic/catalytic processes are also induced (Cantu et al. 2011). To explain the highly efficient translocation of micronutrients from flag leaf to grain enabled by *GPC*, Fe and Zn transporters were analyzed under putative *GPC* expression control. A Heavy Metal ATPase-like (HMA-like) was induced in the presence of *GPC* but was downregulated in *gpc* knock out mutants. HMAs and HMA-like transporters enable the transfer of micronutrients from the chloroplast to the cytoplasm. From here, the released nutrients must reach the phloem. Genes from the ZIP family, which include transporters for Fe (IRT = iron regulated transporter) and Zn (ZRT = zinc regulated transporter), formed the majority of upregulated genes under *GPC* influence. For phloem transport, Fe and Zn must be chelated. In this context, a gene involved in the biosynthesis of the phytosiderophores (PS) nicotianamine (NA), nicotianamine aminotransferase (NAAT), was found at a higher level (Pearce et al. 2014) (Fig. 5). In line with this

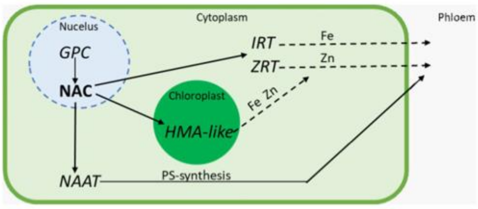

Fig. 5 Regulatory role of the *GPC*-encoded NAC transcription factor for micronutrient translocation. Bold arrows indicate regulated transporters; dashed arrows show the transport of micronutrients. *GPC* = grain protein content gene; NAC = NAC transcription factor; *IRT* = iron-regulated transporter; *ZRT* = zinc-regulated transporter; *HMA-like* = Heavy Metal ATPase-like; *NAAT* = nicotianamine aminotransferase; PS = phytosiderophores

finding, effective uptake and transportation via PS have also been suggested to be responsible for the higher micronutrient concentration in *Aegilops* species compared to *Triticum* species (Neelam et al. 2010). A genome-wide association study with the D genome donor *Ae. tauschii* confirmed the role of PSs by identifying marker–trait associations for iron concentrations. A significant locus was found near a gene involved in PS secretion (Arora et al. 2019). This shows that a subset of genes is controlled by the transcription factor encoding *GPC* gene, which facilitates the translocation of micronutrients. It is a unique example on how valuable wild alleles can be used for biofortification. However, since the *GPC* was not solely responsible for the accumulation of micronutrients in the grain, other genes need to be explored.

The identification of the functional *GPC* gene helps to understand the observed correlations between grain protein, yield and micronutrients explained above. First, it provides a plausible explanation for *T. dicoccoides* being such a promising source for higher grain micronutrient concentrations due to the functional *GPC-B1* allele. However, it remains unclear why there is such a high variation for Zn and Fe between *T. dicoccoides* accessions and how values as low as 15 mg/kg for Fe and 14 mg/kg for Zn can occur despite the presence of these functional alleles. One explanation could be a pronounced environmental influence, e.g., low micronutrient concentration in the soil. However, the wild *GPC-B1* alleles showed a stable contribution across different environments and no interaction between genotype and environment (Distelfeld et al. 2007). Either, these accessions lack the gene, perhaps due to adaption effects to different areas, or other genes associated with micronutrient accumulation may be equally important, which remain to be identified.

Second, the *GPC* gene might be involved in mechanisms leading to the dilution effect suggested earlier. Domesticated varieties contain functional orthologues of the *GPC* allele only on the A and D genomes, which are less efficient in micronutrient translocation from the flag leaf to the grain than the copy on the B genome. Since the *GPC*-mediated translocation is more efficient for Fe than for Zn (Waters et al. 2009; Avni et al. 2014), the lower grain Zn concentration in modern varieties can be explained. However, as stated above *T. dicoccoides* shows a high variation for micronutrients. Assuming that only selected *T. dicoccoides* genotypes were domesticated, there might be a chance that the chosen ones were low accumulators and this trait has been passed on to the domesticates. Regarding a negative effect on yield, the results are contradictory. In a study with recombinant chromosome substitution lines of *T. durum* and *T. dicoccoides*, the functional *GPC-B1* allele did not affect yield (Distelfeld et al. 2007), while in another study a functional allele in hexaploid wheat led to reduced grain weights due to accelerated senescence (Waters et al. 2009). In conclusion, the wild *GPC-B1* allele appears to have a differential effect on different genotypes. When introducing this allele into modern varieties, the genotype-specific yield responses need to be taken into account.

It would be interesting to see if there is an underling geographical pattern indicating environmental factors that have shaped genetic variation enabling biofortification. The bioavailability of micronutrients in cereal grains is limited by phytate, which binds Fe and Zn in insoluble forms and thus makes them unavailable for humans. To date, there is a lack of studies reporting phytate data from wheat wild relatives, which should be addressed in future research along with micronutrient analyses. Overall, the wheat genepool is still poorly explored in terms of diploid species and the timopheevii lineage. These species need to be considered in future research projects. Some of the transport and accumulation pathways of micronutrients in wheat have already been identified. The underlying translocation mechanisms can be targeted in wheat wild relatives to identify mechanisms that can be used in the breeding of biofortified wheat.

Gluten in wheat wild relatives

Gluten is the main form of storage protein in the grain and determines the baking quality. Up to 90% of the protein in the seed are gluten. The gluten protein group includes two components, glutenins and gliadins, which differ in molecular weight and sulfur (S) content (Shewry 2019). Glutenins control dough strength and elasticity but make up only for 10% of the seed storage protein. These polymers are classified according to their molecular weight into low molecular weight glutenin subunit (LMW-GS) and high molecular weight glutenin subunit (HMW-GS). The latter is further subdivided into x and y HMW-GS. Gliadins are monomers comprising the S-rich prolamins α/β and γ gliadins and the S-poor ω-gliadins. Gliadins are responsible

for the viscosity of the dough (Wieser 2007; Biesiekierski 2017; Shewry 2019). The gluten composition in the grain is strongly dependent on the genotype (Wieser 2007). Thus, different *Triticum* species show diversity for different quality and health properties.

Wheat wild relatives harbor rare y-type high molecular weight glutenin subunits

Glutenins are important for the baking quality of the flour and form the backbone of the gluten network. Glutenin content increases in the order of diploid < tetraploid < hexaploid wheats (Geisslitz et al. 2019). The *Glu1* loci on the long arm of chromosome 1 homologous encode two HMW-GS genes, the x- and y-type subunits (Li et al. 2020b; Payne et al. 1980, 1984). The former has a higher molecular weight than the latter. HMW-GS that originate from the D genome influence dough properties the most (Li et al. 2020b). However, y-type subunits are also important for dough quality, although the y-subunit of the A genome (1Ay) is usually not expressed in hexaploid bread wheat (Waines and Payne 1987). Due to the higher number in cysteine residues compared to the x-subunit, the y-subunit is a valuable source for increasing dough quality (Li et al. 2020b).

The variation in HMW-GS decreased with increasing ploidy status during evolution (Xu et al. 2009). The diploid wheat species showed the highest variation in molecules encoding the 1Ay subunit (Hu et al. 2012). The diploid A genome donor of wheat *T. urartu* contained different 1Ay subunit variants (Xu et al. 2009; Caballero et al. 2008; Hu et al. 2010). However, not all 1Ay subunits were expressed (Xu et al. 2009). This pattern was especially found in *T. urartu* accessions from Turkey, Armenia, Iraq and Iran (Waines and Payne 1987). Yet, the variation for 1Ay subunits was characterized by a rare allelic distribution (Caballero et al. 2008). Prediction of the secondary structure of the 1Ay subunit of *T. urartu* showed a potentially beneficial structure for flour processing quality (Hu et al. 2010). *T. boeoticum* may be another source of 1Ay subunit introduction (Waines and Payne 1987). The 1Ay subunit encoded by this species was different from the expressed 1Ay subunit of *T. urartu* (Hu et al. 2012; Jiang et al. 2009). The 1Ay subunit of *T. boeoticum* had a higher molecular mass and was thus longer, which favors the formation of an enhanced gluten network due to interchain bonds (Jiang et al. 2009). Introgression of the *Glu-A1* locus of *T. boeoticum* into bread wheat resulted in an improved dough quality (Rogers et al. 1997). The domesticated einkorn *T. monococcum* contained functional 1Ay subunits, two of which were investigated for their molecular characterization (Guo et al. 2013). One allelic variant showed an additional cysteine residue, which could improve dough quality (Guo et al. 2013). The diversity of 1Ay subunits was higher in *T. boeoticum* and *T. monococcum* than in *T. urartu* (Hu et al. 2012). Thus, the diploid wheats contain useful diversity for enriching dough quality, which should be exploited.

T. dicoccoides contained the highest variation in 1Ay subunits among the tetraploid wheat species (Hu et al. 2012). An active 1Ay subunit in *T. dicoccoides* (GenBank accession: JF519636) resembled the pattern of amino acid sequences of the 1Ay gene found in *T. urartu*, but they differed in their predicted secondary protein structure (Bi et al. 2014). However, in a phylogenic analysis, this 1Ay allele of *T. dicoccoides* clustered together with another *T. dicoccoides* 1Ay subunit (GenBank accession: KC545956). This in turn was introgressed into hexaploid wheat and positively associated with improved dough quality (Wang et al. 2018). The former subunit allele (JF519636) and probably other active 1Ay alleles of *T. dicoccoides* thus harbor putative beneficial quality traits.

The wild tetraploid counterpart *T. araraticum* in the GGAA lineage showed only three different 1Ay subunits (Hu et al. 2012). Two of these 1Ay subunits were similar to *T. boeoticum,* and three were similar to *T. monococccum* (Hu et al. 2012). In *T. timopheevii*, only one 1Ay subunit was found that matched a subunit from *T. monococcum* (Wan et al. 2002; Hu et al. 2012). This variant also occurred in *T. zhukovskyi*. The other variant of the hexaploid GGA^{u}A^{u}A^{m}A^m *T. zhukovskyi* taxon did not occur in one of its putative ancestors (Hu et al. 2012). The variant 1Ay8 subunit in *T. monococcum* was favorable for bread making quality (Guo et al. 2013). *T. araraticum* contained the homologous 1By8 subunit (Hu et al. 2012), which could make *T. araraticum* a potential source for quality improvement.

Wheat wild relatives show valuable y-type HMW-GS that promise improved dough quality. In particular, all diploid ancestors harbor favorable variants. The less explored GGAA lineage and especially *T. zhukovskyi* may be a valuable source for y-type subunits due to its kinship with both diploid A-genome wheat species, *T. urartu* and *T. monococcum*. This is also an advantage over the BBAA and BBAADD wheat species. As the diversity of the active 1Ay subunits decreased during wheat evolution and domestication history, it is of great importance to conserve the wild relatives.

Alpha-gliadins in wild wheat—a safe option for celiac disease patients?

The monomeric gliadins comprise a large gene family with high diversity due to their long evolutionary history. The *Gli* locus comprises genes that encode for gliadins. ω and γ gliadins are encoded by the *Gli1* locus on chromosome 1 homologous. The short arm of chromosome 6 homologues contains the *Gli2* locus, which encodes α/β gliadins (Payne 1986). In addition to its quality-determining properties, gluten has a negative effect on patients with celiac disease (CD). The primary trigger for CD are α-gliadins, whereas the other gliadin groups appear to be less harmful but not entirely safe for some CD patients (Biesiekierski 2017). In the small intestine, gluten is digested (deamidated) and epitopes are formed during this process. These epitopes confer a T cell response that is activated by *HLA-DQ2* and *-DQ8* (HLA = Human leukocyte antigen) and leads to an autoimmune response (reviewed in: Biesiekierski 2017). Alpha-gliadins containing the 33mer peptide are most likely to cause CD. The 33mer peptide comprises the epitopes DQ2.5-glia-α1a and b, DQ2.5-glia-α2 and DQ2.5-glia-α3. The immunodominant 33mer fragment originates from the D genome (Schaart et al. 2020). Therefore, diploid and tetraploid wheats might confer a less toxic immune response.

Domesticated einkorn *T. monococcum* contained more gliadin and thus more gluten than common wheat. This observation was consistent across different locations, leading to the assumption that the higher gluten and gliadin are a characteristic of einkorn wheat (Geisslitz et al. 2019). It is noteworthy that *T. boeoticum*, the wild ancestor of *T. monococcum*, showed higher gliadin content compared to its domesticate (Ozuna and Barro 2018). However, at the molecular level, *T. urartu* and *T. monococcum* lacked some of the toxic epitopes and thus the 33mer peptide (Molberg et al. 2005; Salentijn et al. 2009; Zhang et al. 2015; van Herpen et al. 2006; Ozuna and Barro 2018). Spaenij-Dekking et al. (2005) also found less alpha-gliadins in diploid A genomes. A comparative toxicity study suggested that gliadin of *T. monococcum* would be safer for CD patients than gliadin from *T. aestivum* (Pizzuti et al. 2006). However, *T. monococcum* harbors many different, putatively immunogenic and toxic epitopes; thus, it cannot be considered a CD-safe food only because of the absence of a 33mer peptide (Vaccino et al. 2009; Ozuna et al. 2015). The same is true for *T. urartu*, which lacks the DQ2.5-glia-α2 epitope, but contains DQ2.5-glia-α1a and DQ2.5-glia-α3 CD-triggering epitopes (Zhang et al. 2015; Ruiz-Carnicer et al. 2019). This implies that regarding the quantity and molecular composition of gluten, einkorn is not a safe option for CD patients.

Compared to einkorn, emmer wheat contained less gliadin and gluten, but more than hexaploid bread wheat (Geisslitz et al. 2019). The potential B/G genome donor *Ae. speltoides* also lacked the 33mer peptide (Molberg et al. 2005; Schalk et al. 2017), and conclusively, some of the *T. dicoccon* and *T. durum* samples showed the same phenotype (Molberg et al. 2005). The B genome in tetraploid and hexaploid wheat showed the lowest expression level compared to *Gli A2* and *Gli D2* (Salentijn et al. 2009). Further, it harbored the lowest amount of toxic epitopes (Ozuna et al. 2015). Indeed, *Ae. speltoides*, *Ae. longissima* and *Ae. searsii* showed no abundance of any of the canonical epitopes (Ruiz-Carnicer et al. 2019). However, genes encoding for α-gliadins in *T. dicoccoides* encode toxic epitopes and thus constitute a threat for CD patients (Qi et al. 2006, van den Broeck et al. 2010, Ozuna and Barro 2018). However, the CD-triggering α-gliadin epitopes are most likely retrieved from the A genome (van den Broeck et al. 2010). Alpha-gliadins from the A genome in *T. dicoccoides* contained the T cell epitopes DQ2.5-glia-α1a and DQ2.5-glia-α3, which resembles the pattern in *T. urartu* (Huo et al. 2019; Zhang et al. 2015). In summary, the B genome of tetraploid wheats can be considered the least toxic, but due to the presence of the A genome, tetraploid wheats remain unsuitable for CD patients. However, the content of toxic epitopes varied in tetraploid wheat and decreased as domestication progressed (van den Broeck et al. 2010; Ozuna and Barro 2018). Thus, domesticated species seem to be more suitable for breeding wheat with fewer CD-triggering epitopes. It has to be kept in mind that this approach will generally only help a part of the CD patients, as the variability of toxic epitopes is high and the response to these depends on the individual.

Ae. tauschii formed all of the three harmful epitopes. The DQ2.5-glia-α2 and DQ2.5-glia-α1b epitopes were exclusively assigned to the D genome and were only found in hexaploid wheat, suggesting that this epitope was inherited via the D genome donor (Ruiz-Carnicer et al. 2019; Ozuna and Barro 2018). *Ae. tauschii* showed a high diversity for unique α-gliadin peptide variants, but only accessions from the potential geographic origin of hexaploid wheat hybridization (south-west Caspian Sea) contained the toxic 33mer peptide. On the one hand, this suggests that the CD-triggering epitope in bread wheat originates from this geographic region, but on the other hand, it also underlines that there are many *Ae. tauschii* accessions which might contain less to no toxic peptide variants (Schaart et al. 2020).

The 33mer peptide is the main trigger of CD; however, it seems that even partial epitopes can already cause a reaction in CD patients. Regarding the abundance of canonical epitopes, the D genome donor seems to show the highest profusion, followed by BAD and the A genome donor. The B genome is the least harmful in terms of toxic epitopes (Ruiz-Carnicer et al. 2019). It is still unclear whether the G genome has less toxic α-gliadins to offer, but the timopheevii lineage does not seem to be a promising resource for low α-gliadin epitopes, as the tetraploid *T. araraticum* and *T. timopheevii* potentially have the same A genome donor species as the emmer lineage. Moreover, *T. zhukovskyi* is probably even worse because the A genomes of *T. urartu* and *T. monococcum* are present. Nevertheless, one has to consider that the most toxic 33mer peptide comes from the D genome, which is absent in the timopheevii lineage. Therefore, it might be worthwhile to identify other epitopes there.

Phenolic acids in the wheat genepool

Phenolics occur in free, soluble conjugated and insoluble bound forms, with the latter form being the most abundant (Adom and Liu 2002). The differently bound phenolic acids differ in their bioavailability (Laddomada et al. 2015). Ferulic acid is the major phenolic acid found in wheat (Hernández et al. 2011), and it follows the pattern of the free, soluble conjugated and insoluble bound phenolics at a ratio of 0.1:1:100 (Adom and Liu 2002). Wheat relatives have been investigated for their content in phenolic acids due to their antioxidant capacity.

Data on the phenolics content of wild wheats are rare and contradictory. The wild diploid wheats *T. urartu* and *T. boeoticum* showed higher total polyphenol content (TPC) compared to the domesticated *T. monococcum*, *T. dicoccon* and *T. aestivum*, in which TPCs were almost the same (Brandolini et al. 2015; Yilmaz et al. 2015), while *T. durum* grouped in between (Brandolini et al. 2015). The wild tetraploid emmer revealed the lowest phenolic content among domesticated emmer, *T. durum* and other tetraploid emmer landraces, thus implying inferiority of phenolic content of the wild ancestor of the tetraploid wheats (Laddomada et al. 2017). Some data are available for the domesticated einkorn and emmer. Emmer showed a higher total phenolic content compared to einkorn, which was even lower than the total phenolic content of bread wheat (Serpen et al. 2008). This pattern was also found in the ferulic acid content (Serpen et al. 2008). The opposite was reported by Barański et al. (2020), who measured significantly higher total phenolic acids in einkorn compared to emmer. However, this study only contained one einkorn line (Barański et al. 2020). Both studies revealed contradictory result compared to the equal phenolic content of emmer and einkorn reported by Brandolini et al. (2015). A summary of studies analyzing *T. monococcum*, *T. dicoccon*, *T. durum* and *T. aestivum* with the same method and an overall comparison of studies with different methods were compiled by Shewry and Hey (2015). In the former comparison, einkorn ranked last in the total phenolic acid and ferulic acid content and emmer ranked first, also showing a high variation. In their comparison of different studies, the authors found no distinct differences in the phenolics content across species due to high variability (Shewry and Hey 2015).

In conclusion, no clear pattern can be identified from those studies. This might also be due to different analytical methods applied (Shewry and Hey 2015). Hence, there is no clear evidence that wild wheats possess valuable characteristics for the improvement in phenolic contents in wheat.

The grain quality in an evolutionary context

Evolution describes the gradual process of change and development in populations over time. Thereby, new characteristics or traits develop or disappear. The discussed grain quality characteristics micronutrients and gluten indicate an evolutionary pattern in their variation and accumulation. The main drivers for this phenomenon were a transition from wild to domesticated species and the change in ploidy levels. First of all, the variation for Fe/Zn and 1Ay-glutenin subunits was higher in wild relatives of wheat compared to the domesticated species (Table 1, Fig. 4) (Cakmak et al. 2000, 2004; Peleg et al. 2008; Hu et al. 2012). This implies that during the transformation from wild to cultivated species, those traits were unintentionally altered and fixed, thus reducing the resulting diversity. However, the process of domestication also contributed to more stable grain quality traits, which is especially important for the baking quality. Besides the variation, the level of accumulation of Fe and Zn and α-gliadins also showed an evolutionary trend. The domesticated species harbored lower accumulation potential than the wild relatives. In the case of micronutrients, the gene *GPC-B1*, which turned non-functional during emmer domestication, underlies this phenomenon (Uauy et al. 2006b). High variation and accumulation potential for these grain constituents are thus features of wheat wild relatives that could be exploited in wheat improvement. In the case of phenolics, neither a clear pattern for changes in variation nor for an enrichment of phenolic content in wild and domesticated wheats was identified due to the lack of studies. However, it seems that there might be no evolutionary pattern for this secondary metabolite. Another evolutionary phenomenon that may have affected grain quality is allopolyploidization. The change in ploidy status caused different patterns for gliadin and glutenin during evolution: while an increase in ploidy reduced the gliadin content, the opposite was true for glutenin (Geisslitz et al. 2019).

Due to a lack of information in the scientific literature, the neglected timopheevii lineage should be analyzed regarding its grain quality. As with other wild species, the wild *T. araraticum* would be expected to exhibit high variations in Fe and Zn. Similar α-gliadin derived epitopes were found in *T. urartu* and *T. dicoccoides* and were both obtained from the A genome. Those epitopes would be likely to occur in the timopheevii lineage due to its ancestry with *T. urartu,* and furthermore, *T. zhukovskyi* might additionally contain the epitopes from *T. monococcum*. However, the toxic 33mer peptide is less likely to be represented in this lineage because of the missing hybridization with *Ae. tauschii*. Wild *T. araraticum* could also be a potential source for variation for glutenin subunits, but higher glutenin concentration would be expected in the domesticated tetra- and hexaploid species due to their polyploidization status. These assumptions need scientific evidence, but if they hold true, they could support the concept that evolutionary history can help to identify beneficial species.

Conclusion

The wild relatives of wheat harbor a large genetic diversity for certain grain quality traits such as minerals or gluten content. Additional important grain quality traits such as starch composition, fatty acid composition and non-starch polysaccharide concentration in wheat wild relatives have barely been investigated in the past. Thus, exploring the variation in these compounds in the wild relatives could be interesting topics for future studies. So far, the focus of most quality traits research has been on *T. dicoccoides* because of its straightforward use in bread wheat improvement. Identification of the underlying causative loci and polymorphism can be difficult in wild relatives due to the lack of reference genome sequences. However, ongoing advances in sequencing technology can simplify the development of reference genomes sequences for wheat wild relatives (Avni et al. 2017; Maccaferri et al. 2019; Ling et al. 2018; Walkowiak et al. 2020; Luo et al. 2017; Pont et al. 2019). In addition, the development of a wheat pan-genome sequence can be a valuable strategy in harnessing the genetic diversity of wheat wild relatives (Khan et al. 2020). Furthermore, the advent of genome editing enables de novo domestication strategies for targeted use of crop wild relatives (Fernie and Yan 2019; Zsögön et al. 2017, 2018; Xie and Liu 2021). However, ex situ conservation in genebanks constitutes an important strategy for safeguarding this unexplored diversity and also provides useful information/passport data of the accessions (https://www.genesys-pgr.org). In this context, the diploid taxa should receive more attention, due to their potential for enhancing grain micronutrient concentration as well as y-type HMW-GS. The same applies to the neglected GGAA lineage of wheat, in which quality traits remain to be investigated. In conclusion, further exploring the extended wheat gene pool harbors great potential for wheat diversification and quality improvement.

Online Resource Map: https://de.m.wikipedia.org/wiki/Datei:A_large_blank_world_map_with_oceans_marked_in_blue.PNG.

Acknowledgements This work was supported by a PhD scholarship from the Studienstiftung des deutschen Volkes (FZ). B.K. thanks the Government of Norway (QZA-14/0005) for funding the initiative "Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives" (https://www.cwrdiversity.org/project/pre-breeding/).

Authors' contribution statement FZ compiled and analyzed the reviewed literature and data, structured and wrote the manuscript, and created the figures and table. BK contributed and edited the manuscript. MF contributed and edited the manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. This work was supported by Institute of Agronomy and Plant Breeding I core funding and the Crop Wild Relatives Project (*Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives*), which is supported by the Government of Norway and managed by the Global Crop Diversity Trust [https://www.cwrdiversity.org/]. Moreover, FZ received a PhD scholarship from Studienstiftung des deutschen Volkes.

Declarations

Conflicts of interest The authors declare that they have no conflict of interest.

Ethics approval. The authors declare that the experiments comply with the current laws of Germany.

References

Adonina IG, Goncharov NP, Badaeva ED, Sergeeva EM, Petrash NV, Salina EA (2015) (GAA)n microsatellite as an indicator of the A genome reorganization during wheat evolution and domestication. Comparative Cytogenetics 9(4):533–547. https://doi.org/10.3897/CompCytogen.v9i4.5120

Adom KK, Liu RH (2002) Antioxidant activity of grains. J Agric Food Chem 50(21):6182–6187. https://doi.org/10.1021/jf0205099

Arora S, Cheema J, Poland J, Uauy C, Chhuneja P (2019) Genome-wide association mapping of grain micronutrients concentration in aegilops tauschii. Front Plant Sci 10:54. https://doi.org/10.3389/fpls.2019.00054

Avni R, Nave M, Barad O, Baruch K, Twardziok SO, Gundlach H, Hale I, Mascher M, Spannagl M, Wiebe K, Jordan KW, Golan G, Deek J, Ben-Zvi B, Ben-Zvi G, Himmelbach A, MacLachlan RP, Sharpe AG, Fritz A, Ben-David R, Budak H, Fahima T, Korol A, Faris JD, Hernandez A, Mikel MA, Levy AA, Steffenson B, Maccaferri M, Tuberosa R, Cattivelli L, Faccioli P, Ceriotti A, Kashkush K, Pourkheirandish M, Komatsuda T, Eilam T, Sela H, Sharon A, Ohad N, Chamovitz DA, Mayer KFX, Stein N, Ronen G, Peleg Z, Pozniak CJ, Akhunov ED, Distelfeld A (2017) Wild emmer genome architecture and diversity elucidate

wheat evolution and domestication. Science (New York, NY) 357(6346):93–97. https://doi.org/10.1126/science.aan0032

Avni R, Zhao R, Pearce S, Jun Y, Uauy C, Tabbita F, Fahima T, Slade A, Dubcovsky J, Distelfeld A (2014) Functional characterization of GPC-1 genes in hexaploid wheat. Planta 239(2):313–324. https://doi.org/10.1007/s00425-013-1977-y

Badaeva ED, Keilwagen J, Knüpffer H, Waßermann L, Dedkova OS, Mitrofanova OP, Kovaleva ON, Liapunova OA, Pukhalskiy VA, Özkan H, Graner A, Willcox G, Kilian B (2015) Chromosomal passports provide new insights into diffusion of emmer wheat. PLoS ONE 10(5):e0128556. https://doi.org/10.1371/journal.pone.0128556

Badaeva ED, Konovalov FA, Knüpffer H, Fricano A, Ruban AS, Kehel Z, Zoshchuk SA, Surzhikov SA, Neumann K, Graner A, Hammer K, Filatenko A, Bogaard A, Jones G, Özkan H, Kilian B (2021) Genetic diversity, distribution and domestication history of the neglected GGA t A t genepool of wheat

Baloch FS, Alsaleh A, Shahid MQ, Çiftçi V, Sáenz E, de Miera L, Aasim M, Nadeem MA, Aktaş H, Özkan H, Hatipoğlu R (2017) A Whole genome DArTseq and SNP analysis for genetic diversity assessment in durum wheat from central fertile crescent. PLoS ONE 12(1):e0167821. https://doi.org/10.1371/journal.pone.0167821

Barański M, Lacko-Bartošová M, Rembiałkowska E, Lacko-Bartošová L (2020) The effect of species and cultivation year on phenolic acids content in ancient wheat. Agronomy 10(5):673. https://doi.org/10.3390/agronomy10050673

Bernhardt N, Brassac J, Kilian B, Blattner FR (2017) Dated tribe-wide whole chloroplast genome phylogeny indicates recurrent hybridizations within Triticeae. BMC Evol Biol 17(1):141. https://doi.org/10.1186/s12862-017-0989-9

Bi Z-G, Wu B-H, Hu X-G, Guo X-H, Liu D-C, Zheng Y-L (2014) Identification of an active 1Ay gene from Triticum turgidum ssp. dicoccoides. Czech J Genet Plant Breed. 50:208–215. https://doi.org/10.17221/195/2012-CJGPB

Biesiekierski JR (2017) What is gluten? J Gastroenterol Hepatol 32(Suppl 1):78–81. https://doi.org/10.1111/jgh.13703

Brandolini A, Hidalgo A, Gabriele S, Heun M (2015) Chemical composition of wild and feral diploid wheats and their bearing on domesticated wheats. J Cereal Sci 63:122–127. https://doi.org/10.1016/j.jcs.2015.03.005

Brandolini A, Volante A, Heun M (2016) Geographic differentiation of domesticated einkorn wheat and possible Neolithic migration routes. Heredity 117(3):135–141. https://doi.org/10.1038/hdy.2016.32

Brozynska M, Furtado A, Henry RJ (2016) Genomics of crop wild relatives: expanding the gene pool for crop improvement. Plant Biotechnol J 14(4):1070–1085. https://doi.org/10.1111/pbi.12454

Brunazzi A, Scaglione D, Talini RF, Miculan M, Magni F, Poland J, Enrico Pè M, Brandolini A, Dell'Acqua M (2018) Molecular diversity and landscape genomics of the crop wild relative Triticum urartu across the Fertile Crescent. Plant J 94(4):670–684. https://doi.org/10.1111/tpj.13888

Caballero L, Martín MA, Alvarez JB (2008) Allelic variation for the high- and low-molecular-weight glutenin subunits in wild diploid wheat (Triticum urartu) and its comparison with durum wheats. Aust J Agric Res 59(10):906. https://doi.org/10.1071/AR08065

Cakmak I, Ozkan H, Braun HJ, Welch RM, Romheld V (2000) Zinc and iron concentrations in seeds of wild, primitive, and modern wheats. Food Nutr Bull 21(4):401–403. https://doi.org/10.1177/156482650002100411

Cakmak I, Pfeiffer WH, McClafferty B (2010) REVIEW: biofortification of durum wheat with zinc and iron. Cereal Chemistry Journal 87(1):10–20. https://doi.org/10.1094/CCHEM-87-1-0010

Cakmak I, Torun A, Millet E, Feldman M, Fahima T, Korol A, Nevo E, Braun HJ, Özkan H (2004) Triticum dicoccoides : An important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. Soil Sci Pl Nutr 50(7):1047–1054. https://doi.org/10.1080/00380768.2004.10408573

Cantu D, Pearce SP, Distelfeld A, Christiansen MW, Uauy C, Akhunov E, Fahima T, Dubcovsky J (2011) Effect of the down-regulation of the high Grain Protein Content (GPC) genes on the wheat transcriptome during monocarpic senescence. BMC Genomics 12:492. https://doi.org/10.1186/1471-2164-12-492

Chatzav M, Peleg Z, Ozturk L, Yazici A, Fahima T, Cakmak I, Saranga Y (2010) Genetic diversity for grain nutrients in wild emmer wheat: potential for wheat improvement. Ann Bot 105(7):1211–1220. https://doi.org/10.1093/aob/mcq024

Chhuneja P, Dhaliwal HS, Bains NS, Singh K (2006) Aegilops kotschyi and aegilops tauschii as sources for higher levels of grain iron and zinc. Plant Breed 125(5):529–531. https://doi.org/10.1111/j.1439-0523.2006.01223.x

Dempewolf H, Eastwood RJ, Guarino L, Khoury CK, Müller JV, Toll J (2014) Adapting agriculture to climate change: a global initiative to collect, conserve, and use crop wild relatives. Agroecol Sustain Food Syst 38(4):369–377. https://doi.org/10.1080/21683565.2013.870629

Distelfeld A, Cakmak I, Peleg Z, Ozturk L, Yazici AM, Budak H, Saranga Y, Fahima T (2007) Multiple QTL-effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations. Physiol Plant 129(3):635–643. https://doi.org/10.1111/j.1399-3054.2006.00841.x

Doebley JF, Gaut BS, Smith BD (2006) The molecular genetics of crop domestication. Cell 127(7):1309–1321. https://doi.org/10.1016/j.cell.2006.12.006

Dubcovsky J, Dvorak J (2007) Genome plasticity a key factor in the success of polyploid wheat under domestication. Science 316(5833):1862–1866. https://doi.org/10.1126/science.1143986

El Baidouri M, Murat F, Veyssiere M, Molinier M, Flores R, Burlot L, Alaux M, Quesneville H, Pont C, Salse J (2017) Reconciling the evolutionary origin of bread wheat (Triticum aestivum). New Phytol 213(3):1477–1486. https://doi.org/10.1111/nph.14113

Erba D, Hidalgo A, Bresciani J, Brandolini A (2011) Environmental and genotypic influences on trace element and mineral concentrations in whole meal flour of einkorn (Triticum monococcum L subsp monococcum). J Cereal Sci 54(2):250–254. https://doi.org/10.1016/j.jcs.2011.06.011

Fatima F, McCallum BD, Pozniak CJ, Hiebert CW, McCartney CA, Fedak G, You FM, Cloutier S (2020) Identification of new leaf rust resistance loci in wheat and wild relatives by array-based SNP genotyping and association genetics. Front Plant Sci 11:583738. https://doi.org/10.3389/fpls.2020.583738

Fernie AR, Yan J (2019) De novo domestication: an alternative route toward new crops for the future. Mol Plant 12(5):615–631. https://doi.org/10.1016/j.molp.2019.03.016

Geisslitz S, Longin CFH, Scherf KA, Koehler P (2019) Comparative study on gluten protein composition of ancient (einkorn, emmer and spelt) and modern wheat species (durum and common wheat). Foods (Basel, Switzerland) 8(9):409

Gibson RS, Bailey KB, Gibbs M, Ferguson EL (2010) A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. Food Nutr Bull 31(2 Suppl):S134–S146. https://doi.org/10.1177/15648265100312S206

Golovnina KA, Glushkov SA, Blinov AG, Mayorov VI, Adkison LR, Goncharov NP (2007) Molecular phylogeny of the genus Triticum L. Pl Syst Evol 264(3–4):195–216. https://doi.org/10.1007/s00606-006-0478-x

Gornicki P, Zhu H, Wang J, Challa GS, Zhang Z, Gill BS, Li W (2014) The chloroplast view of the evolution of polyploid wheat. New Phytol 204(3):704–714. https://doi.org/10.1111/nph.12931

Graham RD, Welch RM, Bouis HE (2001) Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: Principles, perspectives and knowledge gaps. In: Advances in agronomy, Academic Press, USA

Graham RD, Welch RM, Saunders DA, Ortiz-Monasterio I, Bouis HE, Bonierbale M, de Haan S, Burgos G, Thiele G, Liria R, Meisner CA, Beebe SE, Potts MJ, Kadian M, Hobbs PR, Gupta R, Twomlow S (2007) Nutritious subsistence food systems. Elsevier, USA

Gross BL, Olsen KM (2010) Genetic perspectives on crop domestication. Trends Plant Sci 15(9):529–537. https://doi.org/10.1016/j.tplants.2010.05.008

Guo X-H, Wu B-H, Hu X-G, Bi Z-G, Wang Z-Z, Liu D-C, ZHENG Y (2013) Molecular characterization of two y-type high molecular weight glutenin subunit alleles 1Ay12 and 1Ay8 from cultivated einkorn wheat (Triticum monococcum ssp monococcum). Gene 516(1):1–7. https://doi.org/10.1016/j.gene.2012.12.037

Gupta PK, Balyan HS, Sharma S, Kumar R (2020) Biofortification and bioavailability of Zn, Fe and Se in wheat: present status and future prospects. Theor Appl Genet. https://doi.org/10.1007/s00122-020-03709-7

Hammer K (1984) Das Domestikationssyndrom. Die Kulturpflanze 32(1):11–34. https://doi.org/10.1007/BF02098682

He F, Pasam R, Shi F, Kant S, Keeble-Gagnere G, Kay P, Forrest K, Fritz A, Hucl P, Wiebe K, Knox R, Cuthbert R, Pozniak C, Akhunova A, Morrell PL, Davies JP, Webb SR, Spangenberg G, Hayes B, Daetwyler H, Tibbits J, Hayden M, Akhunov E (2019) Exome sequencing highlights the role of wild-relative introgression in shaping the adaptive landscape of the wheat genome. Nat Genet 51(5):896–904. https://doi.org/10.1038/s41588-019-0382-2

Henry RJ, Nevo E (2014) Exploring natural selection to guide breeding for agriculture. Plant Biotechnol J 12(6):655–662. https://doi.org/10.1111/pbi.12215

Hernández L, Afonso D, Rodríguez EM, Díaz C (2011) Phenolic compounds in wheat grain cultivars. Plant Foods Human Nutrition 66(4):408–415. https://doi.org/10.1007/s11130-011-0261-1

Heun M (1997) Site of einkorn wheat domestication identified by DNA fingerprinting. Science 278(5341):1312–1314. https://doi.org/10.1126/science.278.5341.1312

Hu XG, Liu J, Zhang L, Wu BH, Hu JL, Liu DC, Zheng YL (2017) Zn and Fe concentration variations of grain and flag leaf and the Relationship with NAM-G1 Gene in Triticum timopheevii (Zhuk) Zhuk ssp timopheevii. Cereal Res Commun 45(3):421–431. https://doi.org/10.1556/0806.45.2017.022

Hu X-G (2012) Characteristics and polymorphism of NAM gene from Aegilops section sitopsis species. Afr J Agric Res. https://doi.org/10.5897/AJAR12.078

Hu X-G, Wu B-H, Bi Z-G, Liu D-C, Zhang L-Q, Yan Z-H, WEI Y, ZHENG Y (2012) Allelic variation and distribution of HMW glutenin subunit 1Ay in Triticum species. Genet Resour Crop Evol 59(4):491–497. https://doi.org/10.1007/s10722-011-9698-1

Hu X-G, Wu B-H, Liu D-C, WEI Y, Gao S-B, ZHENG Y (2013) Variation and their relationship of NAM-G1 gene and grain protein content in Triticum timopheevii Zhuk. J Plant Physiol 170(3):330–337. https://doi.org/10.1016/j.jplph.2012.10.009

Hu X-G, Wu B-H, Yan Z-H, Liu D-C, WEI Y, ZHENG Y (2010) Characterization of a novel 1Ay gene and its expression protein in triticum urartu. Agri Sci China 9(11):1543–1552. https://doi.org/10.1016/S1671-2927(09)60250-9

Huang Q, Börner A, Röder S, Ganal W (2002) Assessing genetic diversity of wheat (Triticum aestivum L) germplasm using microsatellite markers. Theor Appl Genet 105(5):699–707. https://doi.org/10.1007/s00122-002-0959-4

Huang S, Sirikhachornkit A, Su X, Faris J, Gill B, Haselkorn R, Gornicki P (2002b) Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the Triticum/Aegilops complex and the evolutionary history of polyploid wheat. Proc Natl Acad Sci USA 99(12):8133–8138. https://doi.org/10.1073/pnas.072223799

Huo N, Zhu T, Zhang S, Mohr T, Luo M-C, Lee J-Y, Distelfeld A, Altenbach S, Gu YQ (2019) Rapid evolution of α-gliadin gene family revealed by analyzing Gli-2 locus regions of wild emmer wheat. Funct Integr Genomics 19(6):993–1005. https://doi.org/10.1007/s10142-019-00686-z

Iizumi T, Ali-Babiker I-EA, Tsubo M, Tahir ISA, Kurosaki Y, Kim W, Gorafi YSA, Idris AAM, Tsujimoto H (2021) Rising temperatures and increasing demand challenge wheat supply in Sudan. Nat Food 2(1):19–27. https://doi.org/10.1038/s43016-020-00214-4

Jiang Q, Wei Y, Wang F, Wang J, Yan Z-H, Zheng Y (2009) Characterization and comparative analysis of HMW glutenin 1Ay alleles with differential expressions. BMC Plant Biol 9:16. https://doi.org/10.1186/1471-2229-9-16

Johns T, Eyzaguirre PB (2007) Biofortification, biodiversity and diet: a search for complementary applications against poverty and malnutrition. Food Policy 32(1):1–24. https://doi.org/10.1016/j.foodpol.2006.03.014

Joppa LR, Du C, Hart GE, Hareland GA (1997) Mapping gene(s) for grain protein in tetraploid wheat (Triticum turgidum L) using a population of recombinant inbred chromosome lines. Crop Sci. 37(5):1586–1589. https://doi.org/10.2135/cropsci1997.0011183X003700050030x

Kabbaj H, Sall AT, Al-Abdallat A, Geleta M, Amri A, Filali-Maltouf A, Belkadi B, Ortiz R, Bassi FM (2017) Genetic diversity within a global panel of durum wheat (triticum durum) landraces and modern germplasm reveals the history of alleles exchange. Front Plant Sci 8:1277. https://doi.org/10.3389/fpls.2017.01277

Kantar MB, Nashoba AR, Anderson JE, Blackman BK, Rieseberg LH (2017) The genetics and genomics of plant domestication. Bioscience 67(11):971–982. https://doi.org/10.1093/biosci/bix114

Khan AW, Garg V, Roorkiwal M, Golicz AA, Edwards D, Varshney RK (2020) Super-pangenome by integrating the wild side of a species for accelerated crop improvement. Trends Plant Sci 25(2):148–158. https://doi.org/10.1016/j.tplants.2019.10.012

Kilian B, Dempewolf H, Guarino L, Werner P, Coyne C, Warburton ML (2021) Crop science special issue: adapting agriculture to climate change: a walk on the wild side. Crop Sci 61(1):32–36. https://doi.org/10.1002/csc2.20418

Kilian B, Mammen K, Millet E, Sharma R, Graner A, Salamini F, Hammer K, Özkan H (2011) Aegilops. In: Kole C (ed) Wild crop relatives: genomic and breeding resources. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp 1–76

Kilian B, Martin W, Salamini F (2010) Genetic diversity, evolution and domestication of wheat and barley in the fertile crescent. In: Glaubrecht M (ed) Evolution in Action. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp 137–166

Kilian B, Ozkan H, Deusch O, Effgen S, Brandolini A, Kohl J, Martin W, Salamini F (2007a) Independent wheat B and G genome origins in outcrossing Aegilops progenitor haplotypes. Mol Biol Evol 24(1):217–227. https://doi.org/10.1093/molbev/msl151

Kilian B, Ozkan H, Walther A, Kohl J, Dagan T, Salamini F, Martin W (2007b) Molecular diversity at 18 loci in 321 wild and 92 domesticate lines reveal no reduction of nucleotide diversity during Triticum monococcum (Einkorn) domestication: implications for the origin of agriculture. Mol Biol Evol 24(12):2657–2668. https://doi.org/10.1093/molbev/msm192

Konovalov FA, Goncharov NP, Goryunova S, Shaturova A, Proshlyakova T, Kudryavtsev A (2010) Molecular markers based on LTR retrotransposons BARE-1 and Jeli uncover different strata of evolutionary relationships in diploid wheats. Mol Genetics Genom : MGG 283(6):551–563. https://doi.org/10.1007/s00438-010-0539-2

Ladizinsky G (1985) Founder effect in crop-plant evolution. Econ Bot 39(2):191–199. https://doi.org/10.1007/BF02907844

Laddomada B, Caretto S, Mita G (2015) Wheat bran phenolic acids: bioavailability and stability in whole wheat-based foods. Molecules (basel, Switzerland) 20(9):15666–15685. https://doi.org/10.3390/molecules200915666

Laddomada B, Durante M, Mangini G, D'Amico L, Lenucci MS, Simeone R, Piarulli L, Mita G, Blanco A (2017) Genetic variation for phenolic acids concentration and composition in a tetraploid wheat (Triticum turgidum L) collection. Genet Resour Crop Evol 64(3):587–597. https://doi.org/10.1007/s10722-016-0386-z

Li M, Dong L, Li B, Wang Z, Xie J, Qiu D, Li Y, Shi W, Yang L, Wu Q, Chen Y, Lu P, Guo G, Zhang H, Zhang P, Zhu K, Li Y, Zhang Y, Wang R, Yuan C, Liu W, Yu D, Luo M-C, Fahima T, Nevo E, Li H, Liu Z (2020a) A CNL protein in wild emmer wheat confers powdery mildew resistance. New Phytol 228(3):1027–1037. https://doi.org/10.1111/nph.16761

Li Y, Fu J, Shen Q, Yang D (2020) High-molecular-weight glutenin subunits: genetics, structures, and relation to end use qualities. Int J Mol Sci. https://doi.org/10.3390/ijms22010184

Ling H-Q, Ma B, Shi X, Liu H, Dong L, Sun H, Cao Y, Gao Q, Zheng S, Li Y, Yu Y, Du H, Qi M, Li Y, Lu H, Yu H, Cui Y, Wang N, Chen C, Wu H, Zhao Y, Zhang J, Li Y, Zhou W, Zhang B, Hu W, van Eijk MJT, Tang J, Witsenboer HMA, Zhao S, Li Z, Zhang A, Wang D, Liang C (2018) Genome sequence of the progenitor of wheat a subgenome Triticum urartu. Nature 557(7705):424–428. https://doi.org/10.1038/s41586-018-0108-0

Liyana-Pathirana CM, Shahidi F (2006) Importance of insoluble-bound phenolics to antioxidant properties of wheat. J Agric Food Chem 54(4):1256–1264. https://doi.org/10.1021/jf052556h

Luo M-C, Gu YQ, Puiu D, Wang H, Twardziok SO, Deal KR, Huo N, Zhu T, Wang Le, Wang Y, McGuire PE, Liu S, Long H, Ramasamy RK, Rodriguez JC, Van SL, Yuan L, Wang Z, Xia Z, Xiao L, Anderson OD, Ouyang S, Liang Y, Zimin AV, Pertea G, Qi P, Bennetzen JL, Dai X, Dawson MW, Müller H-G, Kugler K, Rivarola-Duarte L, Spannagl M, Mayer KFX, Lu F-H, Bevan MW, Leroy P, Li P, You FM, Sun Q, Liu Z, Lyons E, Wicker T, Salzberg SL, Devos KM, Dvořák J (2017) Genome sequence of the progenitor of the wheat D genome Aegilops tauschii. Nature 551(7681):498–502. https://doi.org/10.1038/nature24486

Luo M-C, Yang Z-L, You FM, Kawahara T, Waines JG, Dvorak J (2007) The structure of wild and domesticated emmer wheat populations, gene flow between them, and the site of emmer domestication. Theor Appl Genet 114(6):947–959. https://doi.org/10.1007/s00122-006-0474-0

Maccaferri M, Harris NS, Twardziok SO, Pasam RK, Gundlach H, Spannagl M, Ormanbekova D, Lux T, Prade VM, Milner SG, Himmelbach A, Mascher M, Bagnaresi P, Faccioli P, Cozzi P, Lauria M, Lazzari B, Stella A, Manconi A, Gnocchi M, Moscatelli M, Avni R, Deek J, Biyiklioglu S, Frascaroli E, Corneti S, Salvi S, Sonnante G, Desiderio F, Marè C, Crosatti C, Mica E, Özkan H, Kilian B, de Vita P, Marone D, Joukhadar R, Mazzucotelli E, Nigro D, Gadaleta A, Chao S, Faris JD, Melo ATO, Pumphrey M, Pecchioni N, Milanesi L, Wiebe K, Ens J, MacLachlan RP, Clarke JM, Sharpe AG, Koh CS, Liang KYH, Taylor GJ, Knox R, Budak H, Mastrangelo AM, Xu SS, Stein N, Hale I, Distelfeld A, Hayden MJ, Tuberosa R, Walkowiak S, Mayer KFX, Ceriotti A, Pozniak CJ, Cattivelli L (2019) Durum wheat genome highlights past domestication signatures and future improvement targets. Nat Genet 51(5):885–895. https://doi.org/10.1038/s41588-019-0381-3

Martínez-Moreno F, Solís I, Noguero D, Blanco A, Özberk İ, Nsarellah N, Elias E, Mylonas I, Soriano JM (2020) Durum wheat in the Mediterranean Rim: historical evolution and genetic resources. Genet Resour Crop Evol 67(6):1415–1436. https://doi.org/10.1007/s10722-020-00913-8

Meyer RS, Purugganan MD (2013) Evolution of crop species: genetics of domestication and diversification. Nat Rev Genet 14(12):840–852. https://doi.org/10.1038/nrg3605

Molberg O, Uhlen AK, Jensen T, Flaete NS, Fleckenstein B, Arentz-Hansen H, Raki M, Lundin KEA, Sollid LM (2005) Mapping of gluten T-cell epitopes in the bread wheat ancestors: implications for celiac disease. Gastroenterology 128(2):393–401. https://doi.org/10.1053/j.gastro.2004.11.003

Monasterio I, Graham RD (2000) Breeding for trace minerals in Wheat. Food Nutr Bull 21(4):392–396. https://doi.org/10.1177/156482650002100409

Moragues M, Moralejo M, Sorrells ME, Royo C (2007) Dispersal of durum wheat [Triticum turgidum L. ssp. turgidum convar. durum (Desf.) MacKey] landraces across the Mediterranean basin assessed by AFLPs and microsatellites. Genet Res Crop Evolution 54(5):1133–1144. https://doi.org/10.1007/s10722-006-9005-8

Morgounov A, Gómez-Becerra HF, Abugalieva A, Dzhunusova M, Yessimbekova M, Muminjanov H, Zelenskiy Y, Ozturk L, Cakmak I (2007) Iron and zinc grain density in common wheat grown in Central Asia. Euphytica 155(1–2):193–203. https://doi.org/10.1007/s10681-006-9321-2

Mori N, Kondo Y, Ishii T, Kawahara T, Valkoun J, Nakamura C (2009) Genetic diversity and origin of timopheevi wheat inferred by chloroplast DNA fingerprinting. Breed Sci 59:571–578

Morrison L (1993) Triticum-Aegilops systematics: taking an integrative approach

Narang D, Kaur S, Steuernagel B, Ghosh S, Bansal U, Li J, Zhang P, Bhardwaj S, Uauy C, Wulff BBH, Chhuneja P (2020) Discovery and characterisation of a new leaf rust resistance gene introgressed in wheat from wild wheat Aegilops peregrina. Sci Rep 10(1):7573. https://doi.org/10.1038/s41598-020-64166-2

Neelam K, Tiwari VK, Rawat N, Tripathi SK, Randhawa GS, Dhaliwal HS (2010) Identification of Aegilops species with higher production of phytosiderophore and iron and zinc uptake under micronutrient-sufficient and -deficient conditions. Plant Genet Res 8(2):132–141. https://doi.org/10.1017/S1479262110000080

Nsarellah N, Amri A, Nachit MM, Bouhssini MEL, Lhaloui S, Lorenzoni C (2003) New durum wheat with Hessian fly resistance from Triticum araraticum and T carthlicum in Morocco. Plant Breed 122(5):435–437. https://doi.org/10.1046/j.1439-0523.2003.00871.x

Oliveira HR, Jacocks L, Czajkowska BI, Kennedy SL, Brown TA (2020) Multiregional origins of the domesticated tetraploid wheats. PLoS ONE 15(1):e0227148. https://doi.org/10.1371/journal.pone.0227148

Oury F-X, Leenhardt F, Rémésy C, Chanliaud E, Duperrier B, Balfourier F, Charmet G (2006) Genetic variability and stability of grain magnesium, zinc and iron concentrations in bread wheat. Eur J Agron 25(2):177–185. https://doi.org/10.1016/j.eja.2006.04.011

Ozkan H, Brandolini A, Pozzi C, Effgen S, Wunder J, Salamini F (2005) A reconsideration of the domestication geography of tetraploid wheats. Theor Appl Genet 110(6):1052–1060. https://doi.org/10.1007/s00122-005-1925-8

Ozkan H, Brandolini A, Torun A, AltIntas S, Eker S, Kilian B, Braun HJ, Salamini F, Cakmak I (2007) Natural variation and identification of microelements content in seeds of einkorn wheat (triticum monococcum). In: Buck HT, Nisi JE, Salomón N (eds)

Wheat production in stressed environments, vol 12. Springer. Netherlands, Dordrecht, pp 455–462

Özkan H, Willcox G, Graner A, Salamini F, Kilian B (2011) Geographic distribution and domestication of wild emmer wheat (Triticum dicoccoides). Genet Resour Crop Evol 58(1):11–53. https://doi.org/10.1007/s10722-010-9581-5

Ozuna CV, Barro F (2018) Characterization of gluten proteins and celiac disease-related immunogenic epitopes in the Triticeae: cereal domestication and breeding contributed to decrease the content of gliadins and gluten. Mol Breed. https://doi.org/10.1007/s11032-018-0779-0

Ozuna CV, Iehisa JCM, Giménez MJ, Alvarez JB, Sousa C, Barro F (2015) Diversification of the celiac disease α-gliadin complex in wheat: a 33-mer peptide with six overlapping epitopes, evolved following polyploidization. Plant J 82(5):794–805. https://doi.org/10.1111/tpj.12851

Payne PI, Holt LM, Jackson EA, Law CN (1984) Wheat storage proteins: their genetics and their potential for manipulation by plant breeding. Phil Trans r Soc Lond B 304(1120):359–371. https://doi.org/10.1098/rstb.1984.0031

Payne PI, Law CN, Mudd EE (1980) Control by homoeologous group 1 chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. Theor Appl Genet 58(3–4):113–120. https://doi.org/10.1007/BF00263101

Pearce S, Tabbita F, Cantu D, Buffalo V, Avni R, Vazquez-Gross H, Zhao R, Conley CJ, Distelfeld A, Dubcovksy J (2014) Regulation of Zn and Fe transporters by the GPC1 gene during early wheat monocarpic senescence. BMC Plant Biol 14:368. https://doi.org/10.1186/s12870-014-0368-2

Peleg Z, Cakmak I, Ozturk L, Yazici A, Jun Y, Budak H, Korol AB, Fahima T, Saranga Y (2009) Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat x wild emmer wheat RIL population. Theor Appl Genet 119(2):353–369. https://doi.org/10.1007/s00122-009-1044-z

Peleg Z, Saranga Y, Yazici A, Fahima T, Ozturk L, Cakmak I (2008) Grain zinc, iron and protein concentrations and zinc-efficiency in wild emmer wheat under contrasting irrigation regimes. Plant Soil 306(1–2):57–67. https://doi.org/10.1007/s11104-007-9417-z

Petersen G, Seberg O, Yde M, Berthelsen K (2006) Phylogenetic relationships of Triticum and Aegilops and evidence for the origin of the A, B, and D genomes of common wheat (Triticum aestivum). Mol Phylogenet Evol 39(1):70–82. https://doi.org/10.1016/j.ympev.2006.01.023

Pizzuti D, Buda A, D'Odorico A, D'Incà R, Chiarelli S, Curioni A, Martines D (2006) Lack of intestinal mucosal toxicity of Triticum monococcum in celiac disease patients. Scand J Gastroenterol 41(11):1305–1311. https://doi.org/10.1080/00365520600699983

Pont C, Leroy T, Seidel M, Tondelli A, Duchemin W, Armisen D, Lang D, Bustos-Korts D, Goué N, Balfourier F, Molnár-Láng M, Lage J, Kilian B, Özkan H, Waite D, Dyer S, Letellier T, Alaux M, Russell J, Keller B, van Eeuwijk F, Spannagl M, Mayer KFX, Waugh R, Stein N, Cattivelli L, Haberer G, Charmet G, Salse J (2019) Tracing the ancestry of modern bread wheats. Nat Genet 51(5):905–911. https://doi.org/10.1038/s41588-019-0393-z

Prasad AS, Halsted JA, Nadimi M (1961) Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. Am J Med 31(4):532–546. https://doi.org/10.1016/0002-9343(61)90137-1

Przewieslik-Allen AM, Wilkinson PA, Burridge AJ, Winfield MO, Dai X, Beaumont M, King J, Yang C-Y, Griffiths S, Wingen LU, Horsnell R, Bentley AR, Shewry P, Barker GLA, Edwards KJ (2021) The role of gene flow and chromosomal instability in shaping the bread wheat genome. Nature Plants 7(2):172–183. https://doi.org/10.1038/s41477-020-00845-2

Purugganan MD, Fuller DQ (2009) The nature of selection during plant domestication. Nature 457(7231):843–848. https://doi.org/10.1038/nature07895

Qi P-F, Yue Y-W, Long H, Wei Y, Yan Z-H, Zheng Y (2006) Molecular characterization of alpha-gliadin genes from wild emmer wheat (Triticum dicoccoides). DNA sequence : J DNA Sequencing Map 17(6):415–421. https://doi.org/10.1080/10425170600931601

Rawat N, Tiwari VK, Singh N, Randhawa GS, Singh K, Chhuneja P, Dhaliwal HS (2009) Evaluation and utilization of Aegilops and wild Triticum species for enhancing iron and zinc content in wheat. Genet Resour Crop Evol 56(1):53–64. https://doi.org/10.1007/s10722-008-9344-8

Rogers WJ, Miller TE, Payne PI, Seekings JA, Sayers EJ, Holt LM, Law CN (1997) Introduction to bread wheat (Triticum aestivum L.) and assessment for bread-making quality of alleles from T. boeoticum Boiss. ssp. thaoudar at Introduction to bread wheat (Triticum aestivum L.) and assessment for Glu-A1 bread-making quality of alleles from T. boeoticum Boiss. ssp. thaoudar at encoding two high-molecular-weight subunits of glutenin. Euphytica 93(1):19–29. doi: https://doi.org/10.1023/A:1002991206350

Ross-Ibarra J, Morrell PL, Gaut BS (2007) Plant domestication, a unique opportunity to identify the genetic basis of adaptation. Proc Natl Acad Sci USA 104(Suppl 1):8641–8648. https://doi.org/10.1073/pnas.0700643104

Ruiz-Carnicer Á, Comino I, Segura V, Ozuna CV, Moreno MdL, López-Casado MÁ, Torres MI, Barro F, Sousa C (2019) Celiac immunogenic potential of α-gliadin epitope variants from triticum and aegilops species. Nutrients. https://doi.org/10.3390/nu11020220

Salamini F, Ozkan H, Brandolini A, Schäfer-Pregl R, Martin W (2002) Genetics and geography of wild cereal domestication in the near east. Nat Rev Genet 3(6):429–441. https://doi.org/10.1038/nrg817

Salentijn EM, Goryunova SV, Bas N, van der Meer IM, van den Broeck HC, Bastien T, Gilissen LJWJ, Smulders MJM (2009) Tetraploid and hexaploid wheat varieties reveal large differences in expression of alpha-gliadins from homoeologous Gli-2 loci. BMC Genomics 10:48. https://doi.org/10.1186/1471-2164-10-48

Schaart JG, Salentijn EMJ, Goryunova SV, Chidzanga C, Esselink DG, Gosman N, Bentley AR, Gilissen LJWJ, Smulders MJM (2020) Exploring the alpha-gliadin locus: the 33-mer peptide with six overlapping coeliac disease epitopes in Triticum aestivum is derived from a subgroup of Aegilops tauschii. Plant J. https://doi.org/10.1111/tpj.15147

Schalk K, Lang C, Wieser H, Koehler P, Scherf KA (2017) Quantitation of the immunodominant 33-mer peptide from α-gliadin in wheat flours by liquid chromatography tandem mass spectrometry. Sci Rep 7:45092. https://doi.org/10.1038/srep45092

Serpen A, Gökmen V, Karagöz A, Köksel H (2008) Phytochemical quantification and total antioxidant capacities of emmer (Triticum dicoccon Schrank) and einkorn (Triticum monococcum L.) wheat landraces. J Agri Food Chem 56(16):7285–7292. https://doi.org/10.1021/jf8010855

Sharma S, Schulthess AW, Bassi FM, Badaeva ED, Neumann K, Graner A, Özkan H, Werner P, Knüpffer H, Kilian B (2021) Introducing beneficial alleles from plant genetic resources into the wheat germplasm. Biology 10(10):982. https://doi.org/10.3390/biology10100982

Shewry PR, Hey S (2015) Do "ancient" wheat species differ from modern bread wheat in their contents of bioactive components? J Cereal Sci 65:236–243. https://doi.org/10.1016/j.jcs.2015.07.014

Shewry P (2019) What is gluten-why is it special? Front Nutr 6:101. https://doi.org/10.3389/fnut.2019.00101

Shiferaw B, Smale M, Braun H-J, Duveiller E, Reynolds M, Muricho G (2013) Crops that feed the world 10 past successes and future challenges to the role played by wheat in global food security. Food Sec. 5(3):291–317. https://doi.org/10.1007/s12571-013-0263-y

Singh K, Chugh V, Sahi GK, Chhuneja P (2012) Wheat: mechanisms and genetic means for improving heat tolerance. In: Tuteja N (ed) Improving crop resistance to abiotic stress. Wiley-Blackwell, Weinheim

Smýkal P, Nelson M, Berger J, von Wettberg E (2018) The impact of genetic changes during crop domestication. Agronomy 8(7):119. https://doi.org/10.3390/agronomy8070119

Spaenij-Dekking L, Kooy-Winkelaar Y, van Veelen P, Drijfhout JW, Jonker H, van Soest L, Smulders MJM, Bosch D, Gilissen LJWJ, Koning F (2005) Natural variation in toxicity of wheat: potential for selection of nontoxic varieties for celiac disease patients. Gastroenterology 129(3):797–806. https://doi.org/10.1053/j.gastro.2005.06.017

Stein AJ (2010) Global impacts of human mineral malnutrition. Plant Soil 335(1–2):133–154. https://doi.org/10.1007/s11104-009-0228-2

Stetter MG, Gates DJ, Mei W, Ross-Ibarra J (2017) How to make a domesticate. Current Biology : CB 27(17):R896–R900. https://doi.org/10.1016/j.cub.2017.06.048

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277(5329):1063–1066. https://doi.org/10.1126/science.277.5329.1063

Trnka M, Rötter RP, Ruiz-Ramos M, Kersebaum KC, Olesen JE, Žalud Z, Semenov MA (2014) Adverse weather conditions for European wheat production will become more frequent with climate change. Nature Clim Change 4(7):637–643. https://doi.org/10.1038/nclimate2242

Uauy C, Brevis JC, Dubcovsky J (2006a) The high grain protein content gene Gpc-B1 accelerates senescence and has pleiotropic effects on protein content in wheat. J Exp Bot 57(11):2785–2794. https://doi.org/10.1093/jxb/erl047

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J (2006) A NAC Gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science (New York, N.Y.) 314(5803):1298–1301. https://doi.org/10.1126/science.1133649

Vaccino P, Becker H-A, Brandolini A, Salamini F, Kilian B (2009) A catalogue of Triticum monococcum genes encoding toxic and immunogenic peptides for celiac disease patients. Mol Genet Genomics : MGG 281(3):289–300. https://doi.org/10.1007/s00438-008-0412-8

van den Broeck H, Hongbing C, Lacaze X, Dusautoir J-C, Gilissen L, Smulders M, van der Meer I (2010) In search of tetraploid wheat accessions reduced in celiac disease-related gluten epitopes. Mol BioSyst 6(11):2206–2213. https://doi.org/10.1039/c0mb00046a

van Herpen TWJM, Goryunova SV, van der Schoot J, Mitreva M, Salentijn E, Vorst O, Schenk MF, van Veelen PA, Koning F, van Soest LJM, Vosman B, Bosch D, Hamer RJ, Gilissen LJWJ, Smulders MJM (2006) Alpha-gliadin genes from the A, B, and D genomes of wheat contain different sets of celiac disease epitopes. BMC Genomics 7:1. https://doi.org/10.1186/1471-2164-7-1

Waines JG, Payne PI (1987) Electrophoretic analysis of the high-molecular-weight glutenin subunits of Triticum monococcum, T. urartu, and the A genome of bread wheat (T. aestivum). Theor Appl Genet 74(1):71–76. https://doi.org/10.1007/BF00290086

Walkowiak S, Gao L, Monat C, Haberer G, Kassa MT, Brinton J, Ramirez-Gonzalez RH, Kolodziej MC, Delorean E, Thambugala D, Klymiuk V, Byrns B, Gundlach H, Bandi V, Siri JN, Nilsen K, Aquino C, Himmelbach A, Copetti D, Ban T, Venturini L, Bevan M, Clavijo B, Koo D-H, Ens J, Wiebe K, N'Diaye A, Fritz AK, Gutwin C, Fiebig A, Fosker C, Fu BX, Accinelli GG, Gardner KA, Fradgley N, Gutierrez-Gonzalez J, Halstead-Nussloch G, Hatakeyama M, Koh CS, Deek J, Costamagna AC, Fobert P, Heavens D, Kanamori H, Kawaura K, Kobayashi F, Krasileva K, Kuo T, McKenzie N, Murata K, Nabeka Y, Paape T, Padmarasu S, Percival-Alwyn L, Kagale S, Scholz U, Sese J, Juliana P, Singh R, Shimizu-Inatsugi R, Swarbreck D, Cockram J, Budak H, Tameshige T, Tanaka T, Tsuji H, Wright J, Wu J, Steuernagel B, Small I, Cloutier S, Keeble-Gagnère G, Muehlbauer G, Tibbets J, Nasuda S, Melonek J, Hucl PJ, Sharpe AG, Clark M, Legg E, Bharti A, Langridge P, Hall A, Uauy C, Mascher M, Krattinger SG, Handa H, Shimizu KK, Distelfeld A, Chalmers K, Keller B, Mayer KFX, Poland J, Stein N, McCartney CA, Spannagl M, Wicker T, Pozniak CJ (2020) Multiple wheat genomes reveal global variation in modern breeding. Nature. https://doi.org/10.1038/s41586-020-2961-x

Wan Y, Wang D, Shewry R, Halford G (2002) Isolation and characterization of five novel high molecular weight subunit of glutenin genes from Triticum timopheevi and Aegilops cylindrica. Theor Appl Genet 104(5):828–839. https://doi.org/10.1007/s00122-001-0793-0

Wang J, Luo M-C, Chen Z, You FM, Wei Y, Zheng Y, Dvorak J (2013) Aegilops tauschii single nucleotide polymorphisms shed light on the origins of wheat D-genome genetic diversity and pinpoint the geographic origin of hexaploid wheat. New Phytol 198(3):925–937. https://doi.org/10.1111/nph.12164

Wang Z, Huang L, Wu B, Hu J, Jiang Z, Qi P, Zheng Y, Liu D (2018) Characterization of an Integrated active glu-1ay allele in common wheat from wild emmer and its potential role in flour improvement. Int J Mol Sci. https://doi.org/10.3390/ijms19040923

Waters BM, Uauy C, Dubcovsky J, Grusak MA (2009) Wheat (Triticum aestivum) NAM proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. J Exp Bot 60(15):4263–4274. https://doi.org/10.1093/jxb/erp257

Weissgerber TL, Milic NM, Winham SJ, Garovic VD (2015) Beyond bar and line graphs: time for a new data presentation paradigm. PLoS Biol 13(4):e1002128. https://doi.org/10.1371/journal.pbio.1002128

Wieser H (2007) Chemistry of gluten proteins. Food Microbiol 24(2):115–119. https://doi.org/10.1016/j.fm.2006.07.004

Xie X, Liu Y-G (2021) De novo domestication towards new crops. National Sci Rev. https://doi.org/10.1093/nsr/nwab033

Xu L-L, Li W, Wei Y, Zheng Y (2009) Genetic diversity of HMW glutenin subunits in diploid, tetraploid and hexaploid Triticum species. Genet Resour Crop Evol 56(3):377–391. https://doi.org/10.1007/s10722-008-9373-3

Yildirim F, Akkaya MS (2006) DNA Fingerprinting and Genetic Characterization of Anatolian Triticum spp using AFLP Markers. Genet Resour Crop Evol 53(5):1033–1042. https://doi.org/10.1007/s10722-004-7938-3

Yilmaz VA, Brandolini A, Hidalgo A (2015) Phenolic acids and antioxidant activity of wild, feral and domesticated diploid wheats. J Cereal Sci 64:168–175. https://doi.org/10.1016/j.jcs.2015.05.005

Zaharieva M, Monneveux P (2014) Cultivated einkorn wheat (Triticum monococcum L subsp monococcum): the long life of a founder crop of agriculture. Genet Resour Crop Evol 61(3):677–706. https://doi.org/10.1007/s10722-014-0084-7

Zhang Y, Luo G, Liu D, Wang D, Yang W, Sun J, Zhang A, Zhan K (2015) Genome-, transcriptome- and proteome-wide analyses of the gliadin gene families in triticum urartu. PLoS ONE 10(7):e0131559. https://doi.org/10.1371/journal.pone.0131559

Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, Huang M, Yao Y, Bassu S, Ciais P, Durand J-L, Elliott J, Ewert F, Janssens IA, Li T, Lin E, Liu Q, Martre P, Müller C, Peng S, Peñuelas J, Ruane AC, Wallach D, Wang T, Wu D, Liu Z, Zhu Y, Zhu Z, Asseng S (2017) Temperature increase reduces global yields of

major crops in four independent estimates. Proc Natl Acad Sci USA 114(35):9326–9331. https://doi.org/10.1073/pnas.1701762114

Zhao FJ, Su YH, Dunham SJ, Rakszegi M, Bedo Z, McGrath SP, Shewry PR (2009) Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. J Cereal Sci 49(2):290–295. https://doi.org/10.1016/j.jcs.2008.11.007

Zohary D (2004) Unconscious selection and the evolution of domesticated plants. Econ Bot 58(1):5–10. https://doi.org/10.1663/0013-0001(2004)058[0005:USATEO]2.0.CO;2

Zohary D, Hopf M, Weiss E (2012) Domestication of plants in the old world: the origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin. Oxford University Press. https://doi.org/10.1093/acprof:osobl/9780199549061.001.0001

Zsögön A, Cermak T, Voytas D, Peres LEP (2017) Genome editing as a tool to achieve the crop ideotype and de novo domestication of wild relatives: Case study in tomato. Plant Sci : Int J Exp Plant Biol 256:120–130. https://doi.org/10.1016/j.plantsci.2016.12.012

Zsögön A, Čermák T, Naves ER, Notini MM, Edel KH, Weinl S, Freschi L, Voytas DF, Kudla J, Peres LEP (2018) De novo domestication of wild tomato using genome editing. Nat Biotechnol. https://doi.org/10.1038/nbt.4272

Online Resource

International Grains Council [IGC] (2020). World Grain Statistics 2016. Available: https://www.igc.int/en/subscriptions/subscription.aspx. Accessed 3 May 2021

3 Phenotyping and identification of target traits for *de novo* domestication of wheat wild relatives

Frederike Zeibig[1], Benjamin Kilian[2], Hakan Özkan[3], Sumitra Pantha[1], Michael Frei[1]

[1] – Department of Agronomy and Crop Physiology, Institute of Agronomy and Plant Breeding I, Justus-Liebig-University, 35392 Giessen, Germany

[2] – Global Crop Diversity Trust, 53113 Bonn, Germany

[3] – Department of Field Crops, Faculty of Agriculture, University of Çukurova, Adana 01330, Turkey

Published in: Energy and Food Security, 13, e497 (2024)

DOI: 10.1002/fes3.497

Supplemental Information can be found in Appendix A.

Received: 17 March 2023 | Revised: 8 August 2023 | Accepted: 10 August 2023
DOI: 10.1002/fes3.497

Food and Energy Security Open Access WILEY

ORIGINAL ARTICLE

Phenotyping and identification of target traits for de novo domestication of wheat wild relatives

Frederike Zeibig[1] | **Benjamin Kilian**[2] | **Hakan Özkan**[3] | **Sumitra Pantha**[1] | **Michael Frei**[1]

[1]Department of Agronomy and Crop Physiology, Institute of Agronomy and Plant Breeding I, Justus-Liebig-University, Giessen, Germany
[2]Global Crop Diversity Trust, Bonn, Germany
[3]Department of Field Crops, Faculty of Agriculture, University of Çukurova, Adana, Turkey

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

Funding information
Development (BOLD)" Project funded by the Government of Norway, Grant/Award Number: *QZA-20/0154*; FZ received a Ph.D. scholarship from the Studienstiftung des Deutschen Volkes.; Institute of Agronomy and Plant Breeding I, Justus-Liebig University Giessen core funding and the "Biodiversity for Opportunities, Livelihoods

Abstract

De novo domestication—the modification of domestication genes in crop wild relatives via genome editing—is an approach for harnessing the beneficial genetic diversity of crop wild relatives. A prerequisite for de novo domestication is phenotyping to identify genetic materials suitable for cultivation in the respective environment. Taxa from the wheat genepool (*Triticum aestivum*, *Triticum durum*, *Triticum monococcum*) are a staple food; these taxa comprise wild relatives of different ploidy levels. The diploid *Triticum boeoticum* and *Triticum urartu* and the tetraploid *Triticum dicoccoides* and *Triticum araraticum* harbor desirable traits such as high grain quality and abiotic and biotic stress tolerance. Hence, they are candidates for de novo domestication. Here, we grew 111 wild wheats and 38 landraces originating predominantly from the Fertile Crescent and six modern wheat cultivars in a field in Giessen, Germany, to evaluate their environmental adaptability to the central European climate and to identify potential candidates and target traits for de novo domestication. We demonstrate that several wild taxa are suitable for cultivation in the central European environment and that they have distinct characteristics that need to be modified during de novo domestication. The normalized difference vegetation index and the thermal time to heading and flowering indicated excellent adaptability of some wheat wild relatives to central European conditions. The values of yield parameters such as grain weight per plant, number of tillers, and thousand kernel weight were lower in the wild wheats than in the landraces. Therefore, these traits should be targeted for improvement during the de novo domestication of wild wheats.

KEYWORDS
de novo domestication, domestication genes, landraces, phenotyping, wheat wild relative

---

1 | INTRODUCTION

Crop diversification is an essential strategy for agricultural production and food systems to ensure food security and nutritious food for a growing population under climate change. Currently, maize, wheat, and rice are the major staples to feed the world, and food systems' homogeneity has increased over the last 50 years (Khoury et al., 2014). Because the global diet relies on a minority of crops compared with the vast array of plant species (Milla & Osborne, 2021; Østerberg et al., 2017), options to re-introduce and increase diversity should be pursued. A common approach for broadening the diversity of modern crops is using crop wild relatives (CWRs) in breeding programs (Dempewolf et al., 2017; Kilian et al., 2021). CWRs harbor vast genetic diversity in terms of biotic and abiotic stress tolerance and resistance and nutritional composition. Usually, the introgression of favorable traits from CWRs is time-consuming, and linkage drag involving undesirable wild genes impairs the performance of the modern crop variety (Bohra et al., 2022; Chen et al., 2019; Dempewolf et al., 2017).

Genome editing of plants enables the more rapid use of CWRs as novel crops, in a process called de novo domestication. It is defined as the introduction of domestication alleles into non-domesticated plants (Fernie & Yan, 2019). De novo domestication using genome editing was successfully applied by Li et al. (2018) and Zsögön et al. (2018), who altered domestication genes affecting fruit size and synchronous flowering in wild tomato (*Solanum pimpinellifolium* L.). Lemmon et al. (2018) edited similar domestication genes in *Physalis pruinosa* L.; thus, de novo domestication via genome editing is feasible. However, there are several obstacles to de novo domestication. Scientific prerequisites for de novo domestication include transformation protocols (Østerberg et al., 2017; van Tassel et al., 2020), annotated reference genome sequences, and identification of suitable orthologs of domestication genes in wild plants (Fernie & Yan, 2019; Østerberg et al., 2017). Agronomic, cultural, social, and legal aspects should also be considered (Østerberg et al., 2017; van Tassel et al., 2020). This implies that genome-edited crops need to be accepted by consumers and comply with relevant laws.

Furthermore, newly domesticated crops need to compete with long-adapted high-yielding crops in terms of land-use and economic value (Luo et al., 2022; Østerberg et al., 2017). The process of obtaining a de novo domesticated crop can be distinguished into several phases, including the editing of (monogenetic) key domestication genes such as loss of shattering (Østerberg et al., 2017), followed by a subsequent breeding program to increase the performance of the novel crop (van Tassel et al., 2020). However, before these steps, the essential prerequisite is phenotyping to determine the new crop's ability to grow in the region(s) of interest, its abiotic and biotic stress resistance or tolerance, its advanced nutritional profile, and its non-toxicity to humans (Østerberg et al., 2017; van Tassel et al., 2020).

Wheat (*Triticum aestivum* L., $2n=6x=42$, BBAADD) is one of the major staples (Langridge et al., 2022). The *Triticum* genepool consists of di-, tetra-, and hexaploid taxa. Two wild diploid and two wild tetraploid *Triticum* taxa have been found in nature: *Triticum urartu* Thumanjan ex Gandilyan ($2n=2x=14$, A^uA^u) and *Triticum boeoticum* Boiss. ($2n=2x=14$, AA, wild einkorn wheat), *Triticum dicoccoides* (Korn. ex. Aschers. & Graebner) Schweinf. ($2n=4x=28$, BBA^uA^u, wild emmer wheat), and *Triticum araraticum* Jakubz. ($2n=4x=28$, GGA^uA^u). Because no wild hexaploid *Triticum* taxa have been found so far, the role of the four wild *Triticum* taxa is of great importance (Zeibig et al., 2021). These taxa have been used as a source of genetic material for introducing biotic and abiotic stress resistance and tolerance into modern bread wheat and are a rich resource of genetic diversity (Dempewolf et al., 2017; Kilian et al., 2011). However, as explained above, introgressing selected traits from wild wheats into modern cultivars can hamper the latter's performance. Because of their strong ability to adapt to marginal environments, their superior grain quality, and their tolerance to pests and diseases, wild wheats could be good candidates for de novo domestication (Yaqoob et al., 2023). They represent important alternatives to modern wheat cultivars from the perspectives of increasing agrobiodiversity, cultivation in marginal locations that are unsuitable for bread and pasta wheat, and supplying nutrients, especially micronutrients.

Before performing de novo domestication, the suitability of wild wheats for cultivation in the respective environment and agricultural system must be evaluated. In terms of their survival, growth, phenology, and productivity, little is known about their adaptability to temperate central European regions. To fill this knowledge gap, we grew a diverse collection of wild diploid and tetraploid wheats, landraces, and modern wheat varieties in a field trial in Giessen, Germany. Our study aimed to identify candidate wild taxa for de novo domestication. For this purpose, we tested the following hypotheses: (i) the temperate central European climate is a suitable environment for wild wheats; and (ii) the phenotypic traits of wild wheats differ from those of modern cultivars and some of these traits should be priority targets for de novo domestication.

2 | METHODS

2.1 | Plant materials

The wild and landrace *Triticum* panel comprised 149 samples (Table 1, Table S1). The wheats were classified

TABLE 1 Taxa, number of accessions sown in the field, classification of biological status and ploidy level of materials used in this study.

Taxon	Number of accessions	Biological status	Ploidy
Triticum boeoticum	7	Wild	2n
Triticum urartu	6	Wild	2n
Triticum dicoccoides	9	Wild	4n
Triticum araraticum	89	Wild	4n
Triticum monococcum	7	Landrace	2n
Triticum dicoccon	14	Landrace	4n
Triticum timopheevii	6	Landrace	4n
Triticum durum	4	Landrace	4n
Triticum aestivum	5	Landrace	6n
Triticum zhukovskyi	2	Landrace	6n
Triticum durum cv.	2	Modern cultivar	4n
Triticum aestivum cv.	4	Modern cultivar	6n

according to Dorofeev et al. (1979) (Kilian et al., 2010). The panel covered four wild taxa and six landrace taxa (Figure 1). The biological status group landrace comprise taxa thar are domesticated descendants of the wild taxa. For comparison, we also grew four modern winter wheat cultivars: cv. Apostel (I.G. Pflanzenzucht), cv. Julius (KWS SAAT SE & Co.), cv. Nordkap (SAATEN UNION), and cv. RGT Reform (RAGT Saaten), and two modern *T. durum* cultivars: cv. Sambadur (Hauptsaaten) and cv. Wintergold (SAATEN UNION).

2.2 | Experimental site

The field (7 m × 36 m) was located at the research station Weilburger Grenze (50°36′ N, 8°39′ E) in Giessen, Germany. The soil was a Fluvic Gleyic Cambisol with 40 kg Nmin/ha. The long-term average annual precipitation was 700 mm/a, and the annual air temperature was 9.7°C, measured from 1981 to 2010. During the experiment, the precipitation was 613.7 mm from planting in November 2020 until harvest in August 2021, and the mean temperature was 9.4°C. The coolest month was February 2021, with a mean monthly temperature of 1.3°C, and the hottest month was June, with a mean monthly temperature of 20.4°C (Figure S1). Climatic data were recorded daily with a weather station at the field station.

2.3 | Plant cultivation

In October 2020, four single seeds per genotype were pre-cultivated in a greenhouse. Seed trays were placed in a vernalization chamber (Weiss Umwelttechnik GmbH, Reiskirchen-Lindenstruth) for stratification for 48 h under an 8-h light/16-h dark photoperiod at 5°C and 60%–70% relative humidity. Afterward, they were transferred to a greenhouse and kept under a 10-h light/14-h dark photoperiod with day/night temperatures of 15°C/10°C. After 1 week, the temperature was decreased to 10°C during the daytime and 5°C during the nighttime, with a 16-h light/8-h dark photoperiod. Within the 2 weeks in the greenhouse, plants reached the two- or three-leaf stage and were then moved outside to acclimatize for 2 days before transplanting into the field in November. The field was arranged in a randomized block design, comprising two blocks with two sub-replicates for each accession in each block.

If only three seedlings emerged, they were separated into two groups (2 + 1), and in case of two seedlings, one was placed in each of the two blocks. The distance between seedlings of the same genotype was 25 cm and that between different genotypes was 50 cm. At the beginning of June, *Drechslera tritici-repentis* was detected, and lice were a major problem at the end of June. Therefore, the plants were sprayed with half-strength solutions of GIGANT® (ADAMA Deutschland GmbH) to treat *D. tritici-repentis* and PIRIMOR® G (ADAMA Deutschland GmbH) to control lice.

2.4 | Phenotyping and physiological measurements

For phenotyping the development of the different taxa, the Zadoks growth scale was used (Zadoks et al., 1974). The heading date was recorded as soon as Zadoks stage 50 (Z50) was reached. The Normalized Difference Vegetation Index (NDVI) was measured for each plant at the heading date at the flag leaf. Duplicate measurements were taken with a Polypen RP140 UVIS Spectroradiometer (PSI

FIGURE 1 Spikelets of wild taxa and spikes of landrace taxa.

Instruments, Drasov, Czech Republic). After that, each plant was covered with a large Crispac microperforated plastic bag (Baumann Saatzuchtbedarf) to avoid cross-fertilization and to ensure that shattered ears and spikelets were retained and could be assigned to a single plant. The majority of the above-ground parts of the plant was covered by a single large micropore bag. Later-emerging side tillers were covered later with smaller bags. The anthesis was recorded as soon as flowering (Z60) was observed. The thermal time (°Cd) needed for heading and anthesis was calculated by adding the daily mean temperature. The base temperature was set to 0°C. Plant height was measured in June after all plants had flowered. Because of the wild relatives' brittle rachis, the height of the stem, from the bottom to the start of the spike, could be measured. The rachis phenotype was recorded at harvest as brittle when all spikelets had disarticulated from the spike at maturity.

2.5 | Analysis of harvest fractions

After harvest, the plants were dried to constant weight at 30°C. The number of tillers was counted, and the spikelets, spikes, and straw of each individual plant were weighed. The spikelets of the landrace and modern wheats are attached to the spike (Figure 1), so the whole spike was weighed. The weights of the wild wheats' spikelets and of the landrace and modern wheats' spikes were recorded as hulled grain weight.

For further analysis, only genotypes with at least three surviving plants were selected, and one sub-replicate in each block was chosen. The spikelets and spikes were threshed using an electric coffee mill. Only the seeds retained in the large microperforated bag, which were most of the seeds produced by the plant, were threshed. The hulless grain weight from the threshed sample was weighed for each plant. To calculate the share of the hulless grain weight to the hulled grain weight, we divided the hulles grain weight by the hulled grain weight. To estimate the total hulless grain weight, the hulled grain weight from the unthreshed samples of the side tillers was multiplied by the share of the hulless grain weight. The thousand kernel weight (TKW) was analyzed by counting three replicates of 50 seeds for each plant. The harvest index (HI) for each plant was calculated as follows: HI = total hulless grain weight ÷ total above-ground biomass (weight of grain, straw, and husks).

We calculated the survival rate of plants to determine how suitable the growing environment was for the different taxa. The survival rate was calculated by dividing the number of plants of each taxon that reached heading and flowering by the number of plants transplanted into the field in November.

2.6 | Statistical analyses

For statistical analysis of the heading and flowering date, stem height, and the number of tillers, one plant in each block had to survive per genotype. Others were excluded from the analysis. If both replicates survived in one block, their mean was calculated for further analysis. For statistical analysis, ANOVA was used to integrate the taxa or biological status as fixed effects and the block as a random effect. Principal component analysis (PCA) was computed. Statistics were performed in R Studio with R version 4.2.0 (www.r-project.org). The packages emmeans, multcomp, and nlme were used for ANOVA, and the packages FactoMineR, factoextra, and corrplot were used for PCA. Figures were generated with ggplot2 and Microsoft Excel.

3 | RESULTS

3.1 | Morphological observations

Different wheat relatives exhibited characteristic panicle stand and spike shapes (Figure S2). During maturity and ripening, spikelet shattering occurred in the wild wheats, and the shattered spikelets and seeds were retained in the microperforated bags. The seeds of the landraces and modern cultivars did not disarticulate from the spike (Figure 1).

3.2 | Survival rate

The wild wheats showed a survival rate (to the heading date) of 98.4%, compared with only 94.3% for the landraces. Among the wild taxa, the diploid *T. boeoticum* and *T. urartu* showed higher survival rates than the tetraploid wild taxa. Among the landraces, *T. monococcum*, *T. timopheevii*, and *T. zhukovskyi* showed a 100% survival rate, whereas *T. durum* (92.3%), *T. aestivum* (88.2%) and *T. dicoccon* (85.2%) lost some plants during the cold period.

3.3 | NDVI

The NDVI differed significantly between the wild taxa and the landraces, but not between the wild taxa and the modern cultivars. Among the wild taxa, the diploid *T. boeoticum* and *T. urartu* performed slightly better than the tetraploid *T. dicoccoides* and *T. araraticum*. Among all the taxa, domesticated einkorn showed the highest NDVI, followed by *T. timopheevii* (Figure S3, Table S3).

3.4 | Heading and anthesis date

All of the samples that survived the winter reached the heading stage (> Z50) and completed anthesis (Z60 and higher). The wild taxa group was the first group to reach the heading stage (Z50) at the beginning of June (mean, 1363 °Cd), followed by the modern cultivars (mean, 1414 °Cd). The landrace group was the latest group to head (mean, 1462 °Cd). The same pattern was found for the thermal time to flowering (Z60). The wild taxa reached anthesis after 1498 °Cd (mean), followed by the modern cultivars (mean, 1556 °Cd) and then the landraces (mean, 1583 °Cd). Compared with the wild and landrace taxa, the modern cultivars showed a more even distribution for both phenological dates. The thermal time to heading (Z50) and flowering (Z60) differed significantly only between the landraces and wild wheat group.

Focusing on the individual taxa in our panel, the wild *T. dicoccoides*, *T. urartu*, and *T. araraticum* were the first to reach the heading stage, whereas *T. monococcum*, *T. timopheevii*, and *T. zhukovskyi* were the last (Figure 2, Table S3). There was no significant difference in the thermal time to heading or to flowering among the taxa in the wild group. The thermal time to flowering in *T. zhukovskyi*

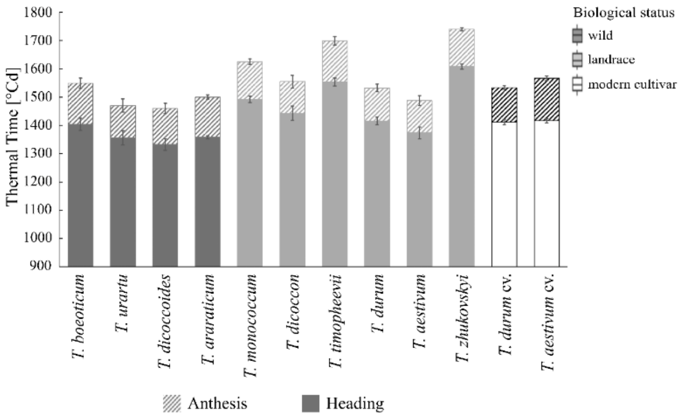

FIGURE 2 Thermal Time [°Cd] required for heading (Z50) and anthesis (Z60) for wild taxa, landraces, and modern cultivars. Bars indicate standard error for each parameter.

and *T. timopheevii* differed significantly from those in all wild and landraces except for *T. monococcum*.

3.5 | Stem height

Plant height is important since it determines the plant's susceptibility to lodging. Modern cultivars showed the lowest average height (63.8 cm), followed by the wild taxa (93.8 cm), and the landraces showed the highest average stem height (104.4 cm). These values differed significantly among the three groups.

Modern bread wheat cultivars were the shortest (61.3 cm) and were significantly shorter than all other taxa except modern durum wheat cultivars. *T. araraticum* was the shortest wild taxon with an average height of 91.5 cm, whereas its domesticate *T. timopheevii s. str.* was the tallest, with an average height of 121.9 cm (Figure 3, Table S2). This follows the pattern that occurred in the groups based on biological status and highlights that the wild taxa are shorter than their domesticated descendants. This was also true for *T. dicoccoides* (93.0 cm) and *T. dicoccon* (106.2 cm) (Figure 3). However, this difference was only significant for *T. araraticum* and *T. timopheevii*. *T. araraticum* was also significantly shorter than the two diploid wild taxa, *T. boeoticum* and *T. urartu*.

3.6 | Number of tillers per plant

Tillers are an important yield component. The median number of tillers per plant was 33 in the wild taxa and 39 in the landraces. The modern cultivars had fewer tillers per plant, with a median of 24. The number of tillers per plant in the landraces differed significantly from those in the wild taxa and modern cultivars. The wild and landrace group showed large variations in the number of tillers. The highest number of tillers was found in *T. monococcum* (58 tillers per plant), followed by its wild ancestor, *T. boeoticum* (45 tillers per plant) (Figure S4, Tables S2 and S3). The number of tillers per plant was significantly different between *T. monococcum* and all other taxa except *T. boeoticum*. This high number was also accompanied by a large variation. This trait generally showed a broad distribution.

3.7 | Hulled and hulless grain weight per plant

Grains of wild and some domesticated taxa are covered by glumes. The hulled grain weight and the hulless grain weight increased from wild to landrace to modern cultivars. The proportion of hulless grain weight out of hulled grain weight increased from 51% in the wild taxa to 74% in the landraces to 87% in the modern cultivars (Figure 3, Table S3). The hulless grain weight, but not the hulled grain weight, differed significantly between the landraces and modern cultivars. However, both of the grain weight traits of the wild taxa differed significantly from those of the landraces and modern cultivars.

T. durum and the modern cultivars of *T. aestivum* cv. had similar hulless grain weights of 55.1 g and 54.5 g per plant, respectively (Figure 4, Table S2). *T. zhukovskyi* had the lowest grain weight per plant with only 7.5 g (Figure 4,

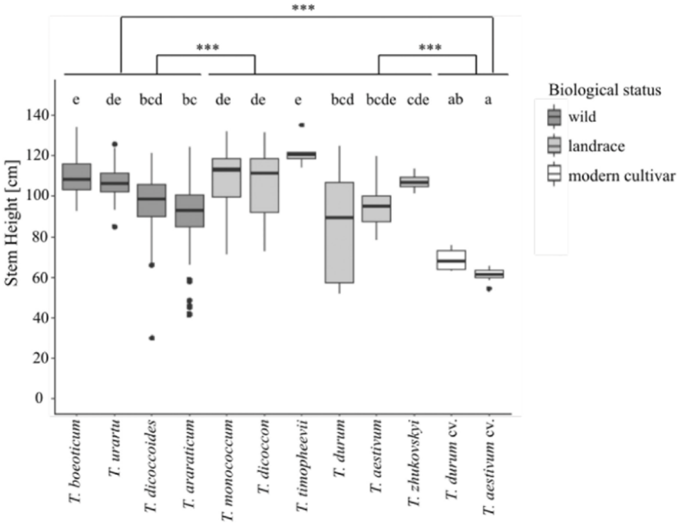

FIGURE 3 Stem height of wild taxa, landraces, and modern cultivars. Shading indicates biological status groups. Asterisks indicate significant differences between biological status groups ($* = p < 0.05$; $** = p < 0.01$; $*** = p < 0.001$), ns. indicates no significant difference, and different letters indicate significant differences among taxa ($p < 0.05$).

FIGURE 4 Hulled grain weight and hulless grain weight per plant for wild taxa, landraces, and modern cultivars. Percentage shows the proportion of hulless grain weight out of hulled grain weight. Bars indicate standard error for each parameter.

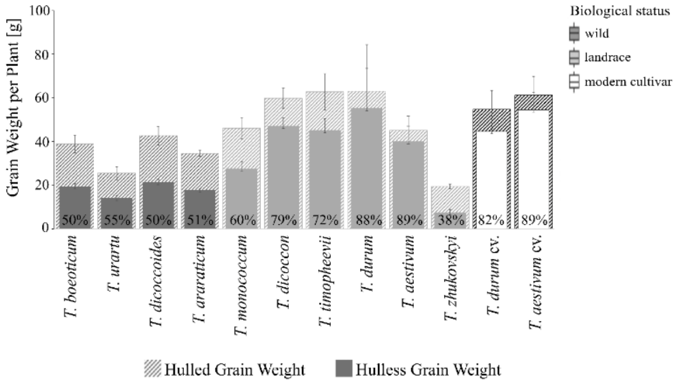

Table S2). The hulless grain weights of the wild taxa were in the lower range from 13.9 to 21.2g and did not differ significantly from each other, but were significantly lower than those of all the modern cultivars and landraces except *T. monococcum* and *T. zhukovskyi*.

3.8 | Thousand kernel weight

The TKW was significantly lower with 24.8g in the wild taxa compared to 34.3g in the landraces and 38g in the modern cultivars. Notably, the TKW did not differ significantly between the landraces and modern cultivars. Focusing on the taxa within the biological status groups, there was a significant difference in TKW between the diploid and tetraploid wild taxa. The TKWs for *T. boeoticum* and *T. urartu* were 13.7g and 11.6g, respectively, much lower than those in *T. dicoccoides* in *T. araraticum* (28.4g and 26.3g, respectively) (Figure 5, Tables S2 and S3). Among the landraces, the diploid *T. monococcum* had a TKW of 20.3g, which was significantly lower than those of the tetra- and hexaploid landraces and cultivars (33.7g). The TKW of tetraploid landraces differed significantly from those of all wild taxa, but not from those of the modern cultivars.

3.9 | Harvest index

The HI is an important characteristic for resource allocation to grain yield and its share of the above-ground biomass. The HI of the wild taxa was 0.26, but higher in the landraces (0.34) and modern cultivars (0.44). The HI differed significantly among the three groups. On the basis of hulless grain weight, *T. zhukovskyi* showed the lowest HI (0.11), which was significantly lower than those of all the other taxa. The HI of the wild taxa and *T. monococcum* ranged from 0.24 to 0.29. The HI of *T. aestivum* landraces (0.41) was similar to that of *T. aestivum* cultivars (0.45) (Figure S5, Tables S2 and S3). Again, the HI of modern cultivars was significantly higher than those of the wild taxa and *T. monococcum*.

3.10 | Connections between phenological, phenotypic, and yield parameters and different taxa

A PCA was performed to see how the taxa clustered on the basis of the variables measured in this study (Figure 6). The majority (89.4%) of the variation in the data set was explained by three principal components (PCs). The first PC (PC1) described 43.5% of the variation in the dataset and was dominated by phenotypic traits such as hulled and hulless grain weight as well as HI, plant height, and tiller number. The second PC accounted for 25.7% of the variation and was described by the thermal times to heading and flowering, that is, by phenological data. The NDVI and number of tillers per plant were the main contributors to the third PC, which explained 20.2% of the variation in the data set. The NDVI had the lowest contribution in the biplot, followed by the number of tillers (Figure 6). The number of tillers clustered on the opposite side of the yield-related traits of hulled grain weight, hulless grain weight, and proportion of hulless grain weight out of hulled grain weight. The HI and the stem height showed an opposing trend.

The wild taxa clustered together, the diploid *T. boeoticum* and *T. urartu* clustered outside the ellipse, and the wild tetraploid *T. araraticum* and *T. dicoccoides* grouped within the ellipse. The landraces were more scattered than the other taxa. *T. dicoccon* and *T. durum* clustered close to *T. aestivum* and the modern cultivars. Notably,

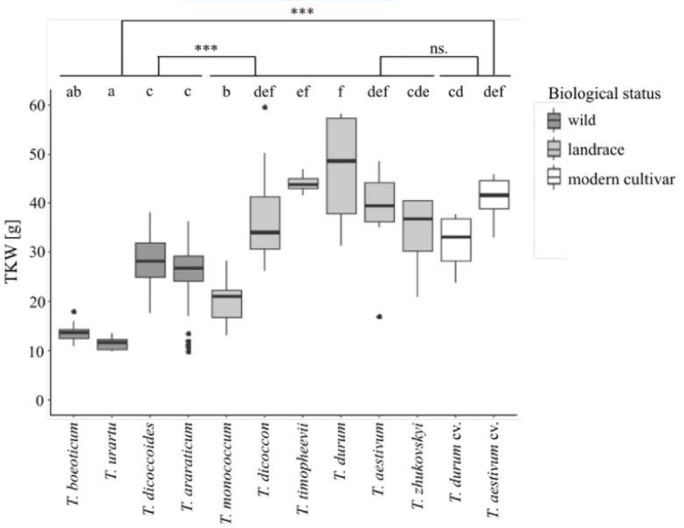

FIGURE 5 Thousand kernel weight (TKW) for wild taxa, landraces, and modern cultivars. Shading indicates biological status groups. Asterisks indicate significant differences between biological status groups (*=$p<0.05$; **=$p<0.01$; ***=$p<0.001$), ns. indicates no significant difference, and different letters indicate significant difference among taxa ($p<0.05$).

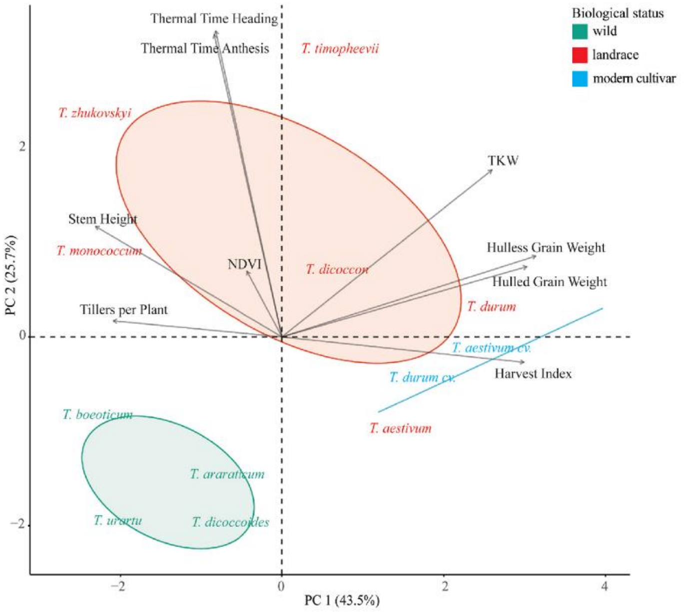

FIGURE 6 Principal component analysis of taxa and phenological, phenotypic, and yield parameters. Taxa are colored according to their biological status. Confidence ellipses are displayed for the biological status.

the modern cultivars of *T. durum* and *T. aestivum* were located near their landraces. This group was found in the proximity of yield-describing traits. The G genome-containing *T. timopheevii* and *T. zhukovskyi* were located distantly from each other (unlike *T. dicoccon* and *T. aestivum*). Still, both were aligned more with the phenological traits of PC2 than with the yield-determining traits of PC1.

4 | DISCUSSION

4.1 | The central European climate is a suitable environment for wild wheats

The survival rate is an important indicator of the adaptability of exotic germplasm to a new environment and the suitability of that environment for their cultivation (van

Tassel et al., 2020). The wheat wild relatives in our study showed high survival rates at the field site, indicating good adaptability. Their adaptation potential is surprising when comparing the climate of their origin to that at the field station. The Fertile Crescent is primarily characterized by a semi-arid to arid climate with winter rains and a hot, dry summer (Jaradat, 2017; Kelley et al., 2015). During the cultivation period in Giessen (Nov 2020–August 2021), May and July had the highest precipitation (Figure S1), but this did not affect the wild wheats too severely. The accessions in our panel had diverse sites of origin. The *T. araraticum* accessions were mainly from Turkey and Iraq, whereas the *T. dicoccoides* accessions were collected from Israel, Turkey, Iran, and Iraq. Consequently, our panel (SI) covered geographic diversity well. Therefore, their good adaptability can also be explained by the diverse environments in their regions of origin, which are shaped by high mountains to drier deserts (Jaradat, 2017). Furthermore, wild wheats are adapted to highly variable yearly precipitation, which ranges from 150 mm to more than 600 mm in their various sites of origin (Willcox, 2005). Considering this, they will likely be suitable for cultivation in other environments in central Europe, although this needs to be tested in field experiments. Another question to be addressed in the future is whether any maternal effect caused differences between wild wheats, which means that the propagation of plants outside their natural habitat would affect seed quality and thus the performance of subsequent generations.

The NDVI is an indicator of chlorophyll content and is related to crop productivity (Thapa et al., 2019) and plant fitness. Typically the NDVI increases until anthesis and peaks at the booting and flowering stages (Hazratkulova et al., 2012; Samborski et al., 2015), consistent with our measurements (Figure S3). The NDVI did not differ significantly between the wild wheat taxa and the modern cultivars, which indicates similar fitness and productivity potential. The chlorophyll content contributes to the yield potential of wheat (Zhang et al., 2009). In this regard, it seems that the wild diploid *T. urartu* and *T. boeoticum* meet the prerequisites for high yield (Figure S3). However, other factors that contribute to yield and productivity, such as grain weight (Figure 4) had lower values in wild wheats than in the modern wheat cultivars in this study. This was expected, because the wild wheats have not been bred for these traits. Due to the similar NDVIs, it can be concluded that the wild taxa are as suitable as the modern wheat cultivars in this environment.

The thermal time can be used to assess the development of plants in response to different environmental factors and genotypic constitutions. Compared with the modern cultivars, the wild taxa tended toward slightly earlier heading (Z50) and flowering (Z60). Still, overall, the developmental stages were reached after a similar amount of degree days (Figure 2). Compared with thermal times reported in other studies, those detected for the wheat panel in this study were highly variable. A short number of degree days was reported for *T. monococcum* and *T. dicoccoides* grown in the USA (Jaradat, 2019). In contrast, modern wheat cultivars grown in Germany took a long time to reach the heading stage (Mahrookashani et al., 2017). Such variation can be attributed to the contributions of vernalization and photoperiod, which strongly influence plant development, but have not been included in the calculations in our study or other studies (McMaster et al., 2008).

Furthermore, in the study of Jaradat (2019), the domesticates were grown as summer crops, thus excluding a vernalization period. However, prolonged times to heading and flowering were found for *T. timopheevii* and *T. zhukovskyi* in the present study (Figure 2). This finding can be attributed to their origin, because they are from a specific part of Georgia (Badaeva et al., 2021). Thus, they might be suitable candidates for stay-green phenotypes and enhanced assimilation. In general, all wild wheats were able to complete their generative development in the central European climate, indicating that they are suitable for cultivation in this area.

4.2 | Wild wheats exhibit phenotypic traits that need to be targeted and modified in de novo domestication

De novo domestication via genome editing offers the opportunity to broaden the diversity of plants for cultivation and accelerate the domestication of wild plants. However, two points must be considered. First, the newly domesticated plant must be accepted by consumers and farmers. For the former, information about genome editing, including its potential and risks, needs to be delivered to, and discussed with, the public, and regulatory policies and frameworks have to be declared. For acceptance by farmers, the crop should be manageable with existing agricultural machinery, such as combine harvesters, to minimize the expense of adaption (van Tassel et al., 2020). Additionally, the de novo domesticated crops must benefit the public and farmers. Secondly, genes underlying unfavorable traits for modern cultivation need to be identified and edited (Fernie et al., 2021; Fernie & Yan, 2019). Most of these genes will comprise domestication-related loci that enable cultivation under the conditions of modern agriculture.

The loss of seed shattering is a major indicator of domestication. Accordingly, we observed the disarticulation of the spikelets of the wild taxa from the spike during ripening. This trait is mainly influenced by the *Brittle rachis 1* and *Brittle rachis 2* (*Btr*) genes on chromosome 3. Their loss of function enables the non-shattering phenotype (Pourkheirandish & Komatsuda, 2022). Thus, those genes should be the first target for de novo domestication of wild wheats.

Hulled grains occur in wheat wild relatives and landraces. This trait hampers threshing and has been selected against during the breeding of bread wheat and durum wheat. In our study, at least 50% of the spikelets consisted of glumes and awns in the wild wheats (Figure 4), resulting in increased biomass, decreased HI, and difficult threshing. The combination of these factors makes this a key target trait because, without straightforward harvesting, de novo domesticated wild wheats will have greater difficulty being accepted by farmers (van Tassel et al., 2020). Traits associated with ease of harvesting have been targeted during domestication, as indicated by an increasing proportion of the hulless grain weight out of hulled grain weight with the advancement in domestication status (Figure 4). Different genes influence the free-threshing character, namely *Q*, *Tg*, and *sog*. The dominant *Q* locus is a major domestication gene in wheat and is located on chromosome 5 (Simons et al., 2006). Threshability is further determined by the *Tenacious glume* (*Tg*) gene on chromosome 2 (Kilian et al., 2009; Sharma et al., 2019; Sood et al., 2009) and *soft glume* (*sog*) (Sood et al., 2009). For the complete free-threshing character, *tg* and *Q* need to be present because they have an additive effect (Sharma et al., 2019). Hulled grains is a major trait that should be targeted in de novo domestication.

Yield parameters distinguished the wild taxa from the landraces and modern cultivars (Figure 6). For successful establishment of de novo domesticated crops derived from CWRs, yield-determining and yield-influencing traits need to be identified and improved. Yield is a complex trait made up of different components (spikes per area, grain weight and size, spike architecture) (Luo et al., 2022; Voss-Fels et al., 2019). In our study, the grain weight parameters (hulled and hulless grain weight) were negatively associated with the number of tillers (Figure 6). Many of the wild samples produced a large number of tillers (Figure S4) but the hulless grain weight was low (Figure 3). This implies that the tillers do not carry a high number of grains per spike or that the TKW was low. Still, those are important traits for productivity in wheat. The number of grains per spike comprises two components; that is, the number of grains per spikelet and the number of spikelets per spike (Kuzay et al., 2019). Different genes have been identified to be responsible for these traits in wheat. The gene *WAPO-A1* on chromosome 7AL positively influences the number of spikelets per spike (Kuzay et al., 2019, 2022).

Similarly, *FT-A2* on chromosome arm 3AS further increases grain number per spike and spike yield (Glenn et al., 2022). A reduced function of *GNI1* (grain number increase) was shown to increase the number of fertile florets per spikelet and thus, the grain number (Sakuma et al., 2019). However, the alleles differ between the wild and domesticated taxa (Glenn et al., 2022; Kuzay et al., 2019, 2022). For de novo domestication, these genes and the corresponding favorable allele(s) should be targeted to increase yield.

The TKW is a highly heritable trait that is an important contributor to grain weight and flour yield (Dziki & Laskowski, 2005). Among the wild samples, the tetraploid taxa showed significantly higher TKW than did the diploid taxa. The TKW of *T. monococcum* was considerably lower than those of the tetraploid landraces and tetraploid wild taxa (Figure 5). This pattern is further supported by other studies that reported TKWs between 20 g and 30 g for *T. monococcum* (Brandolini et al., 2011; Hidalgo & Brandolini, 2017; Kulathunga et al., 2021) and between 33 g and 40 g for *T. dicoccon* (Kulathunga et al., 2021; Marino et al., 2009). Those values are consistent with our observations. Therefore, choosing tetraploid wild taxa for de novo domestication promises a higher yield potential.

The HI represents the partitioning of the grain yield relative to the above-ground biomass and has constantly been improved by breeding to increase grain yield and reduce plant height (Voss-Fels et al., 2019). In our study, the HI was negatively associated with the number of tillers and stem height (Figure 6), which are indicators of large biomass. A tall stem height is a distinctive feature of the wild taxa and landraces (Figure 3). Comparison of stem height data from our study with data reported from different environments revealed some differences. For example, *T. boeoticum* in our study was taller than *T. boeoticum* grown in Iran (Pour-Aboughadareh et al., 2017), whereas *T. urartu* in our study was slightly shorter than *T. urartu* grown in Italy (Talini et al., 2020). This could be attributed to the different environmental conditions (Royo et al., 2014), or to genotypic differences. When performing de novo domestication of wild wheat, reducing plant height is an important target to prevent plants from lodging and increase HI. Genes from the *Rht* dwarfing family would be suitable targets for reducing plant height (Hedden, 2003; Würschum et al., 2017).

The loci proposed here are a first selection of putative target genes for de novo domestication of wild wheats. However, identifying the right orthologs in the wild taxa remains a challenge (Fernie & Yan, 2019; Østerberg et al., 2017). De novo domestication is a chance to repeat the domestication process with the benefits of current knowledge. That implies that we should not abandon diversity at the expense of yield traits. Favorable traits have

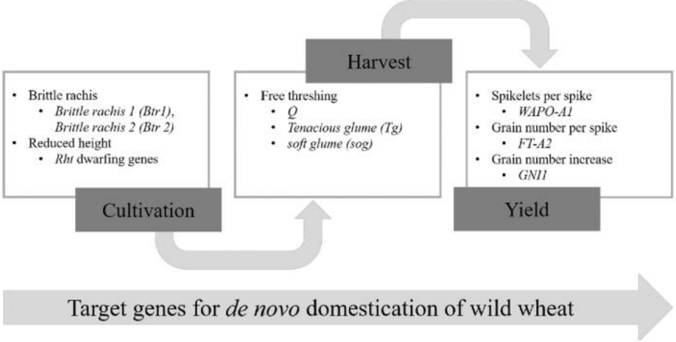

FIGURE 7 Prioritization of genes for a *de novo* domestication of wild wheat. *FT-A2*, *FLOWERING LOCUS T2; GNI1, GRAIN NUMBER INCREASE 1; Rht, Reduced height; WAPO-A1, WHEAT ORTHOLOG OF APO1.*

been lost unintentionally during domestication history and breeding (Fernie & Yan, 2019; van Tassel et al., 2020). Modifying and selecting target traits via genome editing can help to prevent such losses. However, each modification suggested here will interfere with the plant's physiology; therefore, trade-off effects should be screened by phenotyping afterwards. This needs to be emphasized because the domestication loci, such as the *Q* gene, have pleiotropic or additive effects, thus influencing not only the target trait, but also many other traits (Figure 7).

5 | CONCLUSION

The diversity of CWRs provides a resource for ensuring food security under climate change, which can be harnessed by de novo domestication. However, to perform de novo domestication, phenotyping to confirm environmental suitability and the identification of target genes or traits are prerequisites. As a staple food with a diverse genepool, wheat is a good crop for this approach. The wild wheats showed good adaptability to the Central European climate at the field station in Germany with high survival rates and completion of their generative development. The brittle rachis trait in wild wheats should be prioritized and edited first during de novo domestication because it will facilitate cultivation. Genes involved in yield-related traits are also important targets for de novo domestication of wild wheats. Within the wheat taxa, the tetraploid wild wheats showed a higher TKW than did the diploid taxa. Thus, they should be prioritized in future research. These wheats should be grown in different regions to further determine how they are affected by environmental factors. In addition, potential wild wheat candidates should be assessed for novel traits such as tolerance to abiotic stresses and grain quality to choose favorable genotypes for de novo domestication that will increase diversity in the wheat cropping system.

AUTHOR CONTRIBUTIONS

FZ planned the field experiment, took measurements, collected, and analyzed data, created the figures and tables, and structured and wrote the manuscript. BK consulted FZ during the field experiment and contributed to and edited the manuscript. HÖ provided the plant material, and contributed to and edited the manuscript. SP supported FZ in the yield parameter measurements and edited the manuscript. MF conceived the project, contributed to and edited the manuscript.

ACKNOWLEDGMENTS

The authors are grateful to Liane Renno, Erika Schick, Sophia Bohländer, Alena Zink, and Maria Changezi for their assistance during field and lab work. The authors would also like to thank Markus Kolmer and the staff from the field station Weilburger Grenze for their support during field work. The authors are thankful to Dr. Kerstin Neumann for providing the plant material and consultation during the field trial. Open Access funding enabled and organized by Projekt DEAL.

FUNDING INFORMATION

This work was supported by the Institute of Agronomy and Plant Breeding I, Justus-Liebig-University Giessen core funding and the “Biodiversity for Opportunities, Livelihoods and Development (BOLD)” Project funded by the Government of Norway, Grant number *QZA-20/0154*. FZ received a Ph.D. scholarship from the Studienstiftung des Deutschen Volkes.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

All data relevant to this article can be found in the article and the supplementary information.

ETHICS STATEMENT

The authors declare that the experiments comply with the current laws of Germany.

ORCID

Benjamin Kilian https://orcid.org/0000-0002-9145-6980
Michael Frei https://orcid.org/0000-0002-2474-6558

REFERENCES

Badaeva, E. D., Konovalov, F. A., Knüpffer, H., Fricano, A., Ruban, A. S., Kehel, Z., Zoshchuk, S. A., Surzhikov, S. A., Neumann, K., Graner, A., Hammer, K., Filatenko, A., Bogaard, A., Jones, G., Özkan, H., & Kilian, B. (2021). Genetic diversity, distribution and domestication history of the neglected GGA t a t genepool of wheat. *Theoretical and Applied Genetics*, *135*, 755–776. https://doi.org/10.1101/2021.01.10.426084

Bohra, A., Kilian, B., Sivasankar, S., Caccamo, M., Mba, C., McCouch, S. R., & Varshney, R. K. (2022). Reap the crop wild relatives for breeding future crops. *Trends in Biotechnology*, *40*, 412–431. https://doi.org/10.1016/j.tibtech.2021.08.009

Brandolini, A., Hidalgo, A., Plizzari, L., & Erba, D. (2011). Impact of genetic and environmental factors on einkorn wheat (*Triticum monococcum* L. subsp. *monococcum*) polysaccharides. *Journal of Cereal Science*, *53*, 65–72. https://doi.org/10.1016/j.jcs.2010.09.008

Chen, K., Wang, Y., Zhang, R., Zhang, H., & Gao, C. (2019). CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annual Review of Plant Biology*, *70*, 667–697. https://doi.org/10.1146/annurev-arplant-050718-100049

Dempewolf, H., Baute, G., Anderson, J., Kilian, B., Smith, C., & Guarino, L. (2017). Past and future use of wild relatives in crop breeding. *Crop Science*, *57*, 1070–1082. https://doi.org/10.2135/cropsci2016.10.0885

Dorofeev, V., Filatenko, A., Migushova, E., Udaczin, R., & Jakubziner, M. (1979). Wheat. In V. F. Dorofeev & O. N. Korovina (Eds.), *Flora of cultivated plants* (1st ed.). St. Petersburg.

Dziki, D., & Laskowski, J. (2005). Wheat kernel physical properties and milling process. *Acta Agrophysica*, *6*, 59–71.

Fernie, A. R., Alseekh, S., Liu, J., & Yan, J. (2021). Using precision phenotyping to inform *de novo* domestication. *Plant Physiology*, *186*, 1397–1411. https://doi.org/10.1093/plphys/kiab160

Fernie, A. R., & Yan, J. (2019). *De novo* domestication: An alternative route toward new crops for the future. *Molecular Plant*, *12*, 615–631. https://doi.org/10.1016/j.molp.2019.03.016

Glenn, P., Zhang, J., Brown-Guedira, G., DeWitt, N., Cook, J. P., Li, K., Akhunov, E., & Dubcovsky, J. (2022). Identification and characterization of a natural polymorphism in FT-A2 associated with increased number of grains per spike in wheat. *Theoretical and Applied Genetics*, *135*, 679–692. https://doi.org/10.1007/s00122-021-03992-y

Hazratkulova, S., Sharma, R. C., Alikulov, S., Islomov, S., Yuldashev, T., Ziyaev, Z., Khalikulov, Z., Ziyadullaev, Z., & Turok, J. (2012). Analysis of genotypic variation for normalized difference vegetation index and its relationship with grain yield in winter wheat under terminal heat stress. *Plant Breeding*, *131*, 716–721. https://doi.org/10.1111/pbr.12003

Hedden, P. (2003). The genes of the green revolution. *Trends in Genetics*, *19*, 5–9. https://doi.org/10.1016/S0168-9525(02)00009-4

Hidalgo, A., & Brandolini, A. (2017). Nitrogen fertilisation effects on technological parameters and carotenoid, tocol and phenolic acid content of einkorn (*Triticum monococcum* L. subsp. *monococcum*): A two-year evaluation. *Journal of Cereal Science*, *73*, 18–24. https://doi.org/10.1016/j.jcs.2016.11.002

Jaradat, A. A. (2017). Agriculture in the Fertile Crescent: continuity and change under climate change. *CABI Reviews*, *2017*, 1–33. https://doi.org/10.1079/pavsnnr201712034

Jaradat, A. A. (2019). Comparative assessment of einkorn and emmer wheat phenomes: III. Phenology. *Genetic Resources and Crop Evolution*, *66*, 1727–1760. https://doi.org/10.1007/s10722-019-00816-3

Kelley, C. P., Mohtadi, S., Cane, M. A., Seager, R., & Kushnir, Y. (2015). Climate change in the Fertile Crescent and implications of the recent Syrian drought. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 3241–3246. https://doi.org/10.1073/pnas.1421533112

Khoury, C. K., Bjorkman, A. D., Dempewolf, H., Ramirez-Villegas, J., Guarino, L., Jarvis, A., Rieseberg, L. H., & Struik, P. C. (2014). Increasing homogeneity in global food supplies and the implications for food security. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 4001–4006. https://doi.org/10.1073/pnas.1313490111

Kilian, B., Dempewolf, H., Guarino, L., Werner, P., Coyne, C., & Warburton, M. L. (2021). Crop science special issue: Adapting agriculture to climate change: A walk on the wild side. *Crop Science*, *61*, 32–36. https://doi.org/10.1002/csc2.20418

Kilian, B., Mammen, K., Millet, E., Sharma, R., Graner, A., Salamini, F., Hammer, K., & Özkan, H. (2011). Aegilops. In C. Kole (Ed.), *Wild crop relatives: Genomic and breeding resources* (pp. 1–76). Springer.

Kilian, B., Martin, W., & Salamini, F. (2010). Genetic diversity, evolution and domestication of wheat and barley in the Fertile Crescent. In M. Glaubrecht (Ed.), *Evolution in action* (pp. 137–166). Springer.

Kilian, B., Özkan, H., Pozzi, C., & Salamini, F. (2009). Domestication of the Triticeae in the Fertile Crescent. In G. J. Muehlbauer & C. Feuillet (Eds.), *Genetics and genomics of the Triticeae* (pp. 81–119). Springer.

Kulathunga, J., Reuhs, B. L., Zwinger, S., & Simsek, S. (2021). Comparative study on kernelquality and chemical composition of ancient and modern wheat species: Einkorn, emmer, spelt, and hard red spring wheat. *Food*, *10*, 761. https://doi.org/10.3390/foods10040761

Kuzay, S., Lin, H., Li, C., Chen, S., Woods, D. P., Zhang, J., Lan, T., von Korff, M., & Dubcovsky, J. (2022). WAPO-A1 is the causal gene of the 7AL QTL for spikelet number per spike in wheat. *PLoS Genetics*, *18*, e1009747. https://doi.org/10.1371/journal.pgen.1009747

Kuzay, S., Xu, Y., Zhang, J., Katz, A., Pearce, S., Su, Z., Fraser, M., Anderson, J. A., Brown-Guedira, G., DeWitt, N., Peters Haugrud, A., Faris, J. D., Akhunov, E., Bai, G., & Dubcovsky, J. (2019). Identification of a candidate gene for a QTL for spikelet number per spike on wheat chromosome arm 7AL by high-resolution genetic mapping. *Theoretical and Applied*

Genetics, *132*, 2689–2705. https://doi.org/10.1007/s00122-019-03382-5

Langridge, P., Alaux, M., Almeida, N. F., Ammar, K., Baum, M., Bekkaoui, F., Bentley, A. R., Beres, B. L., Berger, B., Braun, H.-J., Brown-Guedira, G., Burt, C. J., Caccamo, M. J., Cattivelli, L., Charmet, G., Civáň, P., Cloutier, S., Cohan, J.-P., Devaux, P. J., … Zhang, X. (2022). Meeting the challenges facing wheat production: The strategic research agenda of the global wheat initiative. *Agronomy*, *12*, 2767. https://doi.org/10.3390/agronomy12112767

Lemmon, Z. H., Reem, N. T., Dalrymple, J., Soyk, S., Swartwood, K. E., Rodriguez-Leal, D., van Eck, J., & Lippman, Z. B. (2018). Rapid improvement of domestication traits in an orphan crop by genome editing. *Nature Plants*, *4*, 766–770. https://doi.org/10.1038/s41477-018-0259-x

Li, T., Yang, X., Yu, Y., Si, X., Zhai, X., Zhang, H., Dong, W., Gao, C., & Xu, C. (2018). Domestication of wild tomato is accelerated by genome editing. *Nature Biotechnology*, *36*, 1160–1163. https://doi.org/10.1038/nbt.4273

Luo, G., Najafi, J., Correia, P. M. P., Trinh, M. D. L., Chapman, E. A., Østerberg, J. T., Thomsen, H. C., Pedas, P. R., Larson, S., Gao, C., Poland, J., Knudsen, S., DeHaan, L., & Palmgren, M. (2022). Accelerated domestication of new crops: Yield is key. *Plant & Cell Physiology*, *63*, 1624–1640. https://doi.org/10.1093/pcp/pcac065

Mahrookashani, A., Siebert, S., Hüging, H., & Ewert, F. (2017). Independent and combined effects of high temperature and drought stress around anthesis on wheat. *Journal of Agronomy and Crop Science*, *203*, 453–463. https://doi.org/10.1111/jac.12218

Marino, S., Tognetti, R., & Alvino, A. (2009). Crop yield and grain quality of emmer populations grown in central Italy, as affected by nitrogen fertilization. *European Journal of Agronomy*, *31*(4), 233–240. https://doi.org/10.1016/j.eja.2009.08.002

McMaster, G. S., White, J. W., Hunt, L. A., Jamieson, P. D., Dhillon, S. S., & Ortiz-Monasterio, J. I. (2008). Simulating the influence of vernalization, photoperiod and optimum temperature on wheat developmental rates. *Annals of Botany*, *102*, 561–569. https://doi.org/10.1093/aob/mcn115

Milla, R., & Osborne, C. P. (2021). Crop origins explain variation in global agricultural relevance. *Nature Plants*, *7*, 598–607. https://doi.org/10.1038/s41477-021-00905-1

Østerberg, J. T., Xiang, W., Olsen, L. I., Edenbrandt, A. K., Vedel, S. E., Christiansen, A., Landes, X., Andersen, M. M., Pagh, P., Sandøe, P., Nielsen, J., Christensen, S. B., Thorsen, B. J., Kappel, K., Gamborg, C., & Palmgren, M. (2017). Accelerating the domestication of new crops: Feasibility and approaches. *Trends in Plant Science*, *22*, 373–384. https://doi.org/10.1016/j.tplants.2017.01.004

Pour-Aboughadareh, A., Mahmoudi, M., Moghaddam, M., Ahmadi, J., Mehrabi, A. A., & Alavikia, S. S. (2017). Agro-morphological and molecular variability in *Triticum boeoticum* accessions from Zagros Mountains, Iran. *Genetic Resources and Crop Evolution*, *64*, 545–556. https://doi.org/10.1007/s10722-016-0381-4

Pourkheirandish, M., & Komatsuda, T. (2022). Grain disarticulation in wild wheat and barley. *Plant & Cell Physiology*, *63*, 1584–1591. https://doi.org/10.1093/pcp/pcac091

Royo, C., Nazco, R., & Villegas, D. (2014). The climate of the zone of origin of Mediterranean durum wheat (*Triticum durum* Desf.) landraces affects their agronomic performance. *Genetic Resources and Crop Evolution*, *61*, 1345–1358. https://doi.org/10.1007/s10722-014-0116-3

Sakuma, S., Golan, G., Guo, Z., Ogawa, T., Tagiri, A., Sugimoto, K., Bernhardt, N., Brassac, J., Mascher, M., Hensel, G., Ohnishi, S., Jinno, H., Yamashita, Y., Ayalon, I., Peleg, Z., Schnurbusch, T., & Komatsuda, T. (2019). Unleashing floret fertility in wheat through the mutation of a homeobox gene. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 5182–5187. https://doi.org/10.1073/pnas.1815465116

Samborski, S. M., Gozdowski, D., Walsh, O. S., Lamb, D. W., Stępień, M., Gacek, E. S., & Drzazga, T. (2015). Winter wheat genotype effect on canopy reflectance: Implications for ssing NDVI for in-season nitrogen topdressing recommendations. *Agronomy Journal*, *107*, 2097–2106. https://doi.org/10.2134/agronj14.0323

Sharma, J. S., Running, K. L. D., Xu, S. S., Zhang, Q., Peters Haugrud, A. R., Sharma, S., McClean, P. E., & Faris, J. D. (2019). Genetic analysis of threshability and other spike traits in the evolution of cultivated emmer to fully domesticated durum wheat. *Molecular Genetics and Genomics*, *294*, 757–771. https://doi.org/10.1007/s00438-019-01544-0

Simons, K. J., Fellers, J. P., Trick, H. N., Zhang, Z., Tai, Y.-S., Gill, B. S., & Faris, J. D. (2006). Molecular characterization of the major wheat domestication gene Q. *Genetics*, *172*, 547–555. https://doi.org/10.1534/genetics.105.044727

Sood, S., Kuraparthy, V., Bai, G., & Gill, B. S. (2009). The major threshability genes soft glume (sog) and tenacious glume (Tg), of diploid and polyploid wheat, trace their origin to independent mutations at non-orthologous loci. *Theoretical and Applied Genetics*, *119*, 341–351. https://doi.org/10.1007/s00122-009-1043-0

Talini, R. F., Brandolini, A., Miculan, M., Brunazzi, A., Vaccino, P., Pè, M. E., & Dell'Acqua, M. (2020). Genome-wide association study of agronomic and quality traits in a world collection of the wild wheat relative *Triticum urartu*. *The Plant Journal*, *102*, 555–568. https://doi.org/10.1111/tpj.14650

Thapa, S., Rudd, J. C., Xue, Q., Bhandari, M., Reddy, S. K., Jessup, K. E., Liu, S., Devkota, R. N., Baker, J., & Baker, S. (2019). Use of NDVI for characterizing winter wheat response to water stress in a semi-arid environment. *Journal of Crop Improvement*, *33*, 633–648. https://doi.org/10.1080/15427528.2019.1648348

van Tassel, D. L., Tesdell, O., Schlautman, B., Rubin, M. J., DeHaan, L. R., Crews, T. E., & Streit Krug, A. (2020). New food crop domestication in the age of gene editing: Genetic, agronomic and cultural change remain co-evolutionarily entangled. *Frontiers in Plant Science*, *11*, 789. https://doi.org/10.3389/fpls.2020.00789

Voss-Fels, K. P., Stahl, A., & Hickey, L. T. (2019). Q&a: Modern crop breeding for future food security. *BMC Biology*, *17*, 18. https://doi.org/10.1186/s12915-019-0638-4

Willcox, G. (2005). The distribution, natural habitats and availability of wild cereals in relation to their domestication in the near east: Multiple events, multiple centres. *Vegetation History and Archaeobotany*, *14*, 534–541. https://doi.org/10.1007/s00334-005-0075-x

Würschum, T., Langer, S. M., Longin, C. F. H., Tucker, M. R., & Leiser, W. L. (2017). A modern green revolution gene for reduced height in wheat. *The Plant Journal*, *92*, 892–903. https://doi.org/10.1111/tpj.13726

Yaqoob, H., Tariq, A., Bhat, B. A., Bhat, K. A., Nehvi, I. B., Raza, A., Djalovic, I., Prasad, P. V., & Mir, R. A. (2023). Integrating genomics and genome editing for orphan crop improvement: A bridge between orphan crops and modern agriculture system. *GM Crops & Food*, *14*, 1–20. https://doi.org/10.1080/21645698.2022.2146952

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research*, *14*, 415–421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

Zeibig, F., Kilian, B., & Frei, M. (2021). The grain quality of wheat wild relatives in the evolutionary context. *Theoretical and Applied Genetics*, *135*, 4029–4048. https://doi.org/10.1007/s00122-021-04013-8

Zhang, K., Zhang, Y., Chen, G., & Tian, J. (2009). Genetic analysis of grain yield and leaf chlorophyll content in common wheat. *Cereal Research Communications*, *37*, 499–511. https://doi.org/10.1556/CRC.37.2009.4.3

Zsögön, A., Čermák, T., Naves, E. R., Notini, M. M., Edel, K. H., Weinl, S., Freschi, L., Voytas, D. F., Kudla, J., & Peres, L. E. P. (2018). *De novo* domestication of wild tomato using genome editing. *Nature Biotechnology*, *36*, 1211–1216. https://doi.org/10.1038/nbt.4272

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Zeibig, F., Kilian, B., Özkan, H., Pantha, S., & Frei, M. (2024). Phenotyping and identification of target traits for de novo domestication of wheat wild relatives. *Food and Energy Security*, *13*, e497. https://doi.org/10.1002/fes3.497

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

Frederike Zeibig[1], Benjamin Kilian[2], Hakan Özkan[3], Sumitra Pantha[1], Michael Frei[1]

[1] – Department of Agronomy and Crop Physiology, Institute of Agronomy and Plant Breeding I, Justus-Liebig-University, 35392 Giessen, Germany

[2] – Global Crop Diversity Trust, 53113 Bonn, Germany

[3] – Department of Field Crops, Faculty of Agriculture, University of Çukurova, Adana 01330, Turkey

Published in: Journal of the Science of Food and Agriculture, 104, 4400-4410 (2024)

DOI: 10.1002/jsfa.13328

Supplemental Information can be found in Appendix B.

Research Article

Received: 25 October 2023 Revised: 18 January 2024 Accepted article published: 19 January 2024

(wileyonlinelibrary.com) DOI 10.1002/jsfa.13328

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,

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

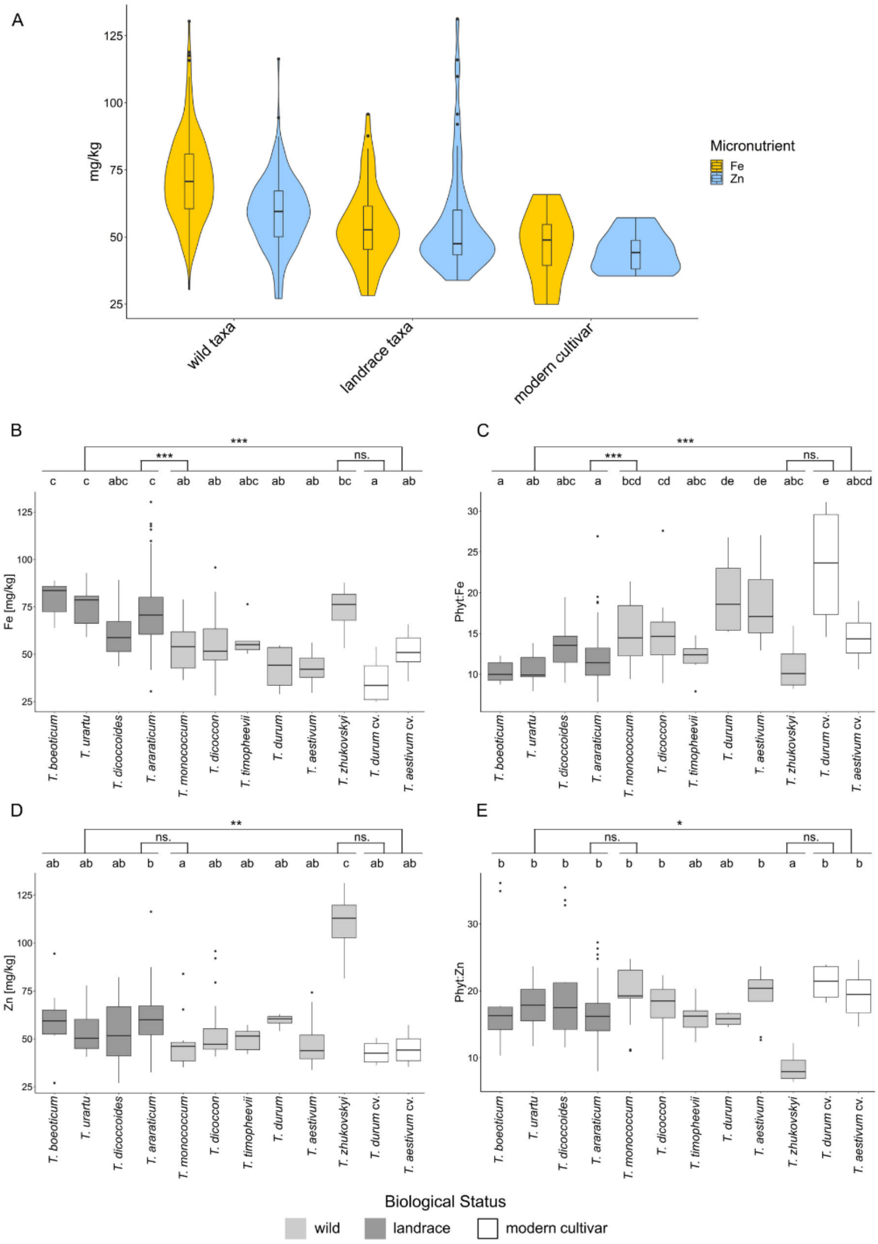

Figure 1. Fe and Zn grain concentrations and their bioavailability in wild taxa, landraces and modern cultivars. (A) Distribution of micronutrient concentrations according to biological status (wild, landrace or modern cultivar). (B) Fe grain concentrations (mg kg^{-1}) in wild taxa, landrace taxa and 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 ($P < 0.05$).

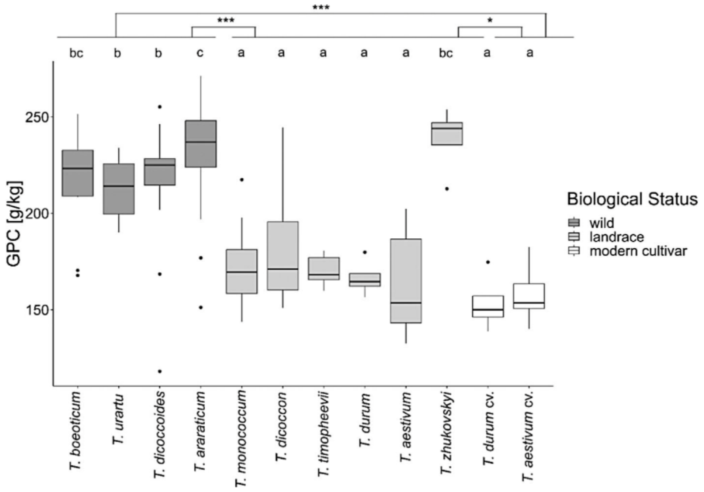

Figure 2. Grain protein content (GPC) of wild taxa, landraces and modern cultivars. Asterisks indicate significant differences between groups according to their biological status (*$P < 0.05$: **$P < 0.01$; ***$P < 0.001$); ns, no significant difference; Different letters indicate significant differences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

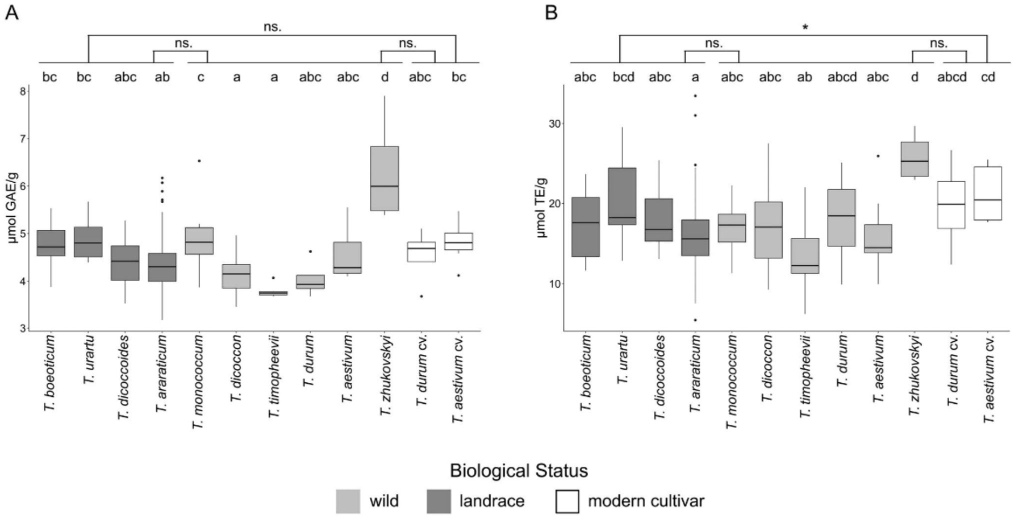

Figure 3. Antioxidant activity of wild taxa, landraces and modern cultivars. (A) Total phenolic content [µmol gallic acid equivalents (GAE) g^{-1}] of wild taxa, landraces and modern cultivars. (B) Oxygen radical absorption capacity [µmol Trolox equivalents (TE) g^{-1}] of wild taxa, landraces and modern 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 ($P < 0.05$).

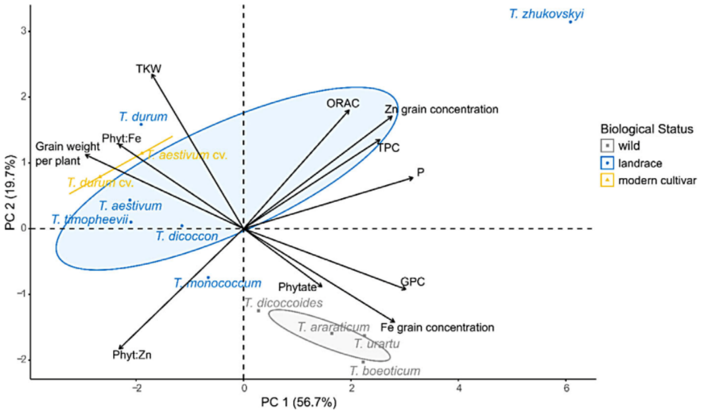

Figure 4. Principal component analysis of taxa and grain quality and yield parameters of thousand kernel weight (TKW) and grain weight per plant. Confidence ellipses are shown for biological status. TPC denotes total phenolic content and ORAC denotes oxygen radical absorbance capacity.

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}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 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 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.

ACKNOWLEDGEMENTS

This work was supported by core funding from the Institute of Agronomy and Plant Breeding I, Justus Liebig University Giessen and the 'Biodiversity for Opportunities, Livelihoods and Development' (BOLD) Project funded by the Government of Norway, Grant number *QZA-20/0154*. FZ received a PhD scholarship from the Studienstiftung des Deutschen Volkes. We thank Liane Renno, Erika Schick, Alena Zink and Maria Changezi for their assistance during laboratory work. Open Access funding enabled and organized by Projekt DEAL.

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.

REFERENCES

1 Erenstein O, Jaleta M, Mottaleb KA, Sonder K, Donovan J and Braun H-J, Global trends in wheat production, consumption and trade, in *Wheat Improvement*, ed. by Reynolds MP and Braun H-J. Springer International Publishing, Cham, pp. 47–66 (2022).
2 Igrejas G, Ikeda TM and Guzmán C eds, *Wheat Quality for Improving Processing and Human Health*. Springer International Publishing, Cham (2020).
3 Langridge P, Alaux M, Almeida NF, Ammar K, Baum M, Bekkaoui F *et al.*, Meeting the challenges facing wheat production: the strategic research agenda of the global wheat initiative. *Agronomy* **12**:2767 (2022).
4 Govindan V, Michaux KD and Pfeiffer WH, Nutritionally enhanced wheat for food and nutrition security, in *Wheat Improvement*, ed. by Reynolds MP and Braun H-J. Springer International Publishing, Cham, pp. 195–214 (2022).
5 Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R and Peña RJ, Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *J Cereal Sci* **46**:293–307 (2007).
6 Velu G, Ortiz-Monasterio I, Cakmak I, Hao Y and Singh RP, Biofortification strategies to increase grain zinc and iron concentrations in wheat. *J Cereal Sci* **59**:365–372 (2014).
7 Mateo Anson N, van den Berg R, Havenaar R, Bast A and Haenen GRM, Ferulic acid from aleurone determines the antioxidant potency of wheat grain (*Triticum aestivum* L.). *J Agric Food Chem* **56**:5589–5594 (2008).
8 Badaeva ED, Konovalov FA, Knüpffer H, Fricano A, Ruban AS, Kehel Z *et al.*, Genetic diversity, distribution and domestication history of the neglected GGA t a t genepool of wheat. *Theor Appl Genet* **135**: 755–776 (2021).
9 Zeibig F, Kilian B and Frei M, The grain quality of wheat wild relatives in the evolutionary context. *Theor Appl Genet* **135**:4029–4048 (2021).
10 Badaeva ED, Ruban AS, Zoshchuk SA, Surzhikov SA, Knüpffer H and Kilian B, Molecular cytogenetic characterization of *Triticum timopheevii* chromosomes provides new insight on genome evolution of *T. Zhukovskyi*. *Pl Syst Evol* **302**:943–956 (2016).
11 Sharma S, Schulthess AW, Bassi FM, Badaeva ED, Neumann K, Graner A *et al.*, Introducing beneficial alleles from plant genetic resources into the wheat germplasm. *Biology* **10**:982 (2021).
12 Gupta PK, Balyan HS, Sharma S and Kumar R, Biofortification and bioavailability of Zn, Fe and Se in wheat: present status and future prospects. *Theor Appl Genet* **134**:1–35 (2020).
13 Tadesse W, Sachez-Garcia M, Assefa SG, Amri A, Bishaw Z, Ogbonnaya F *et al.*, Genetic gains in wheat breeding and its role in feeding the world. *Crop Breed Genet Genom* **1**:e190005 (2019).
14 Cakmak I, Ozkan H, Braun HJ, Welch RM and Romheld V, Zinc and iron concentrations in seeds of wild, primitive, and modern wheats. *Food Nutr Bull* **21**:401–403 (2000).
15 Biel W, Jaroszewska A, Stankowski S, Sobolewska M and Kępińska-Pacelik J, Comparison of yield, chemical composition and farino-

graph properties of common and ancient wheat grains. *Eur Food Res Technol* **247**:1525–1538 (2021).

16 Chatzav M, Peleg Z, Ozturk L, Yazici A, Fahima T, Cakmak I *et al.*, Genetic diversity for grain nutrients in wild emmer wheat: potential for wheat improvement. *Ann Bot* **105**:1211–1220 (2010).

17 Geisslitz S, Longin CFH, Scherf KA and Koehler P, Comparative study on gluten protein composition of ancient (einkorn, emmer and spelt) and modern wheat species (durum and common wheat). *Foods* **8**: 409 (2019).

18 Ciaffi M, Dominici L, Lafiandra D and Porceddu E, Seed storage proteins of wild wheat progenitors and their relationships with technological properties. *Hereditas* **116**:315–322 (1992).

19 Monaghan JM, Snape JW, Chojecki AJS and Kettlewell PS, The use of grain protein deviation for identifying wheat cultivars with high grain protein concentration and yield. *Euphytica* **122**:309–317 (2001).

20 Yilmaz VA, Brandolini A and Hidalgo A, Phenolic acids and antioxidant activity of wild, feral and domesticated diploid wheats. *J Cereal Sci* **64**:168–175 (2015).

21 Nocente F, Galassi E, Taddei F, Natale C and Gazza L, Ancient Caucasian wheats: a contribution for sustainable diets and food diversity. *Foods* **11**:1209 (2022).

22 Laddomada B, Durante M, Mangini G, D'Amico L, Lenucci MS, Simeone R *et al.*, Genetic variation for phenolic acids concentration and composition in a tetraploid wheat (*Triticum turgidum* L.) collection. *Genet Resour Crop Evol* **64**:587–597 (2017).

23 Bohra A, Kilian B, Sivasankar S, Caccamo M, Mba C, McCouch SR *et al.*, Reap the crop wild relatives for breeding future crops. *Trends Biotechnol* **40**:412–431 (2022).

24 Chen K, Wang Y, Zhang R, Zhang H and Gao C, CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annu Rev Plant Biol* **70**:667–697 (2019).

25 Kilian B, Dempewolf H, Guarino L, Werner P, Coyne C and Warburton ML, Crop science special issue: adapting agriculture to climate change: a walk on the wild side. *Crop Sci* **61**:32–36 (2021).

26 Fernie AR and Yan J, De novo domestication: An alternative route toward new crops for the future. *Mol Plant* **12**:615–631 (2019).

27 Fernie AR, Alseekh S, Liu J and Yan J, Using precision phenotyping to inform de novo domestication. *Plant Physiol* **186**:1397–4011 (2021).

28 Xie X and Liu Y-G, *De novo* domestication towards new crops. *Natl Sci Rev* **8**:nwab033 (2021).

29 Luo G, Najafi J, Correia PMP, Trinh MD, Chapman EA, Østerberg JT *et al.*, Accelerated domestication of new crops: yield is key. *Plant Cell Physiol* **63**:1624–1640 (2022).

30 Østerberg JT, Xiang W, Olsen LI, Edenbrandt AK, Vedel SE, Christiansen A *et al.*, Accelerating the domestication of new crops: feasibility and approaches. *Trends Plant Sci* **22**:373–384 (2017).

31 van Tassel DL, Tesdell O, Schlautman B, Rubin MJ, DeHaan LR, Crews TE *et al.*, New food crop domestication in the age of gene editing: genetic, agronomic and cultural change remain co-evolutionarily entangled. *Front Plant Sci* **11**:789 (2020).

32 Zeibig F, Kilian B, Özkan H, Pantha S and Frei M, Phenotyping and identification of target traits for de novo domestication of wheat wild relatives. *Food Energy Secur* **13**:e497 (2023). https://doi.org/10.1002/fes3.497.

33 Vaintraub IA and Lapteva NA, Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. *Anal Biochem* **175**:227–230 (1988).

34 Gericke S and Kurmies B, Die kolorimetrische Phosphorsäurebestimmung mit Ammonium-Vanadat-Molybdat und ihre Anwendung in der Pflanzenanalyse. *Z. Pflanzenernähr Düng. Bodenk* **59**:235–247 (1952).

35 Beta T, Nam S, Dexter JE and Sapirstein HD, Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. *Cereal Chem* **82**:390–393 (2005).

36 Ainsworth EA and Gillespie KM, Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat Protoc* **2**:875–877 (2007).

37 Gillespie KM, Chae JM and Ainsworth EA, Rapid measurement of total antioxidant capacity in plants. *Nat Protoc* **2**:867–870 (2007).

38 Bálint AF, Kovács G, Erdei L and Sutka J, Comparison of the Cu, Zn, Fe, Ca and Mg contents of the grains of wild, ancient and cultivated wheat species. *Cereal Res Commun* **29**:375–382 (2001).

39 Cakmak I, Torun A, Millet E, Feldman M, Fahima T, Korol A *et al.*, *Triticum dicoccoides*: an important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. *Soil Sci Plant Nutr* **50**:1047–1054 (2004).

40 Xu Y, An D, Li H and Xu H, Review: breeding wheat for enhanced micronutrients. *Can J Plant Sci* **91**:231–237 (2011).

41 Gibson RS, Bailey KB, Gibbs M and Ferguson EL, A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food Nutr Bull* **31**:S134–S146 (2010).

42 Gibson RS, Donovan UM and Heath AL, Dietary strategies to improve the iron and zinc nutriture of young women following a vegetarian diet. *Plant Foods Hum Nutr* **51**:1–16 (1997).

43 Magallanes-López AM, Hernandez-Espinosa N, Velu G, Posadas-Romano G, Ordoñez-Villegas VMG, Crossa J *et al.*, Variability in iron, zinc and phytic acid content in a worldwide collection of commercial durum wheat cultivars and the effect of reduced irrigation on these traits. *Food Chem* **237**:499–505 (2017).

44 Salunke R, Rawat N, Neelam K, Tiwari VK, Randhawa GS, Dhaliwal HS *et al.*, Effect of grain hardness on bioavailability of iron in wheat as determined using the coupled invitro digestion/Caco-2 model. *LWT* **59**:433–438 (2014).

45 Hussain S, Maqsood M and Miller L, Bioavailable zinc in grains of bread wheat varieties of Pakistan. *Cereal Res Commun* **40**:62–73 (2012).

46 Erdal I, Yilmaz A, Taban S, Eker S, Torun B and Cakmak I, Phytic acid and phosphorus concentrations in seeds of wheat cultivars grown with and without zinc fertilization. *J Plant Nutr* **25**:113–127 (2002).

47 Guzmán C, Ibba MI, Álvarez JB, Sissons M and Morris C, Wheat quality, in *Wheat Improvement*, ed. by Reynolds MP and Braun H-J. Springer International Publishing, Cham, pp. 177–193 (2022).

48 Keçeli A, Kaplan Evlice A, Pehlivan A, Şanal T and Zencirci N, Bread-making properties of einkorn wheat (*Triticum monococcum* ssp. *monococcum*) and its suitability for whole wheat bread production. *Cereal Res Commun* **49**:625–637 (2021).

49 Kulathunga J, Reuhs BL, Zwinger S and Simsek S, Comparative study on kernel quality and chemical composition of ancient and modern wheat species: einkorn, emmer, spelt and hard red spring wheat. *Foods* **10**:761 (2021).

50 Peleg Z, Saranga Y, Yazici A, Fahima T, Ozturk L and Cakmak I, Grain zinc, iron and protein concentrations and zinc-efficiency in wild emmer wheat under contrasting irrigation regimes. *Plant Soil* **306**: 57–67 (2008).

51 Cabas-Lühmann P, Arriagada O, Matus I, Marcotuli I, Gadaleta A and Schwember AR, Comparison of durum with ancient tetraploid wheats from an agronomical, chemical, nutritional, and genetic standpoints: a review. *Euphytica* **219**:61 (2023).

52 Brandolini A, Hidalgo A, Gabriele S and Heun M, Chemical composition of wild and feral diploid wheats and their bearing on domesticated wheats. *J Cereal Sci* **63**:122–127 (2015).

53 Simmonds NW, The relation between yield and protein in cereal grain. *J Sci Food Agric* **67**:309–315 (1995).

54 Shewry PR and Hey S, Do "ancient" wheat species differ from modern bread wheat in their contents of bioactive components? *J Cereal Sci* **65**:236–243 (2015).

55 Giambanelli E, Ferioli F, Koçaoglu B, Jorjadze M, Alexieva I, Darbinyan N *et al.*, A comparative study of bioactive compounds in primitive wheat populations from Italy, Turkey, Georgia, Bulgaria and Armenia. *J Sci Food Agric* **93**:3490–3501 (2013).

56 Li L, Shewry PR and Ward JL, Phenolic acids in wheat varieties in the HEALTHGRAIN diversity screen. *J Agric Food Chem* **56**:9732–9739 (2008).

57 Peng T, Jiang X, Wang D, Zhang M, Liu X, Hao M *et al.*, Population structure and genetic diversity of *Triticum araraticum* Jakubz. And *Triticum timopheevii* Zhuk. *Genet Resour Crop Evol* **70**:1799–1815 (2023).

58 Rawat N, Tiwari VK, Singh N, Randhawa GS, Singh K, Chhuneja P *et al.*, Evaluation and utilization of *Aegilops* and wild *Triticum* species for enhancing iron and zinc content in wheat. *Genet Resour Crop Evol* **56**:53–64 (2009).

59 Afzal M, Sielaff M, Distler U, Schuppan D, Tenzer S and Longin CFH, Reference proteomes of five wheat species as starting point for future design of cultivars with lower allergenic potential. *NPJ Sci Food* **7**:9 (2023).

60 Vaccino P, Becker H-A, Brandolini A, Salamini F and Kilian B, A catalogue of *Triticum monococcum* genes encoding toxic and immunogenic peptides for celiac disease patients. *Mol Gen Genomics* **281**: 289–300 (2009).

5 Discussion

5.1 Wheat diversity and domestication

Domestication of wheat took place in the Fertile Crescent and is thus wheat's center of diversity (Feldman & Levy, 2023). However, the Fertile Crescent is a vast area and therefore wheat species are shaped by topographic and climatic influences. The *Triticum* genepool comprised four wild taxa, two diploid taxa *T. urartu* and *T. boeoticum* and two tetraploid taxa *T. dicoccoides* and *T. araraticum*. Except *T. urartu*, the wild taxa are related to domesticated landrace taxa *T. monococcum*, *T. dicoccon* and/or *T. durum* and *T. timopheevii*, respectively. The hexaploid *T. aestivum* and *T. zhukovskyi* form domesticated hexaploid landrace taxa. Research on the origin and evolution of the GGAA wheats *T. araraticum*, *T. timopheevii* and *T. zhukovsyki* recently received interest and knowledge is building up (Badaeva *et al.*, 2021; Zeibig *et al.*, 2022; Feldman & Levy, 2023). In conclusion the *Triticum* taxa offers a broad diversity that can be explored for a *de novo* domestication.

5.2 Grain quality in wild wheat

The wild wheat taxa have not underwent breeding and thus harbor an untapped diversity (Zeibig *et al.*, 2022). The meta-analysis revealed an elevated grain Zn concentration and greater diversity in the wheat wild relatives compared to domesticated wheats. Only little variation was found in the grain Fe concentration between wild and domesticated taxa (Zeibig *et al.*, 2022). Elevated grain micronutrient concentration was associated with the functional *GPC*-gene conferring accelerated senescence and higher GPC (Distelfeld *et al.*, 2007; Waters *et al.*, 2009; Avni *et al.*, 2014). Functionality of the *GPC*-gene was found in *T. dicoccoides*, but not in domesticated *T. durum* and *T. aestivum* (Uauy *et al.*, 2006). Thus GPC, together with grain Fe and Zn concentration, was included in the grain quality analysis of Chapter 4. Rare y - high molecular glutenin subunits showed a great diversity in wild and domesticated diploid wheat species and wild Emmer (Zeibig *et al.*, 2022). Focusing on gliadins in gluten, all wheat taxa harbored harmful epitopes that could confer celiac disease (Zeibig *et al.*, 2022). In conclusion, wheat relatives can improve the baking quality but are not suitable for celiac disease patients. The review in Chapter 2 further revealed a scarce and contradictory data situation regarding the TPC in wheat and its wild relatives and no clear evidence for beneficial TPC in wheat wild relatives was found (Zeibig *et al.*, 2022). Therefore, this parameter was also included in the grain quality analysis in Chapter 4. Comparable for the current knowledge on the evolutionary history, only few data was available for the grain quality of GGAA wheats. The grain quality of *T. urartu* has received little attention to date (Talini *et al.*, 2020). Therefore, the potential of GGAA wheats and *T. urartu* for grain micronutrient concentration, gluten composition and TPC remain to be elucidated. The gradual transformation from a wild to a domesticated wheat influenced the grain quality shown by grain micronutrient concentration and GPC. To support this observation, the wild wheats were grown and analyzed in comparison with their domesticated landrace taxa. If proven to be true, those taxa are ideal for *de novo* domestication to bypass the effect of domestication on grain quality.

5.3 Phenotyping wild wheat for a *de novo* domestication

5.3.1 The *Triticum* genepool offers taxa that is suitable for the cultivation in a central European climate

De novo domestication offers the opportunity to broaden agrobiodiversity in the crop cultivation system (Fernie & Yan, 2019; Luo *et al.*, 2022; Hebelstrup *et al.*, 2023). Therefore, the suitability of the taxa in the target environment and agricultural system needs to be assessed (Fernie & Yan, 2019; Fernie *et al.*, 2021; Luo *et al.*, 2022). Information on the performance and suitability of wild wheats and GGAA wheats is rare and thus, this step was crucial to explore the domestication and agricultural potential of wheat wild relatives.

Originally from the Fertile Crescent, the wild wheats were grown out of their respective origin with cooler temperatures and higher precipitation (Willcox, 2005; Jaradat, 2017; Zeibig *et al.*, 2024a). Despite these conditions, the wild wheats showed a good adaptability to the central European climate indicated by phenological and physiological observations (Zeibig *et al.*, 2024a). This observations aligned with the findings that wild crop progenitors already harbor beneficial ecophysiological traits and can thus be utilized as agricultural crops (Gómez-Fernández *et al.*, 2024). Hence, this first assessment verifies the suitability of wild wheats to the central European climate.

5.3.2 Wild wheats exhibit phenotypic traits that need to be targeted and modified in *de novo* domestication

Traits in wild wheats distinguished them clearly from domesticated landraces and modern cultivars. These traits were grouped related to cultivation for the loss of shattering and reduced height, harvest for the ease of threshing and yield for increase in spikelets per spike and grain number per spike. Associated target genes for the respective traits were proposed to be edited in a *de novo* domestication (Zeibig *et al.*, 2024a).

The loss of shattering is a key domestication trait controlled by the *Brittle rachis* gene *1* and *2* (*Btr1*, *Btr2*), that was first discovered in barley (Pourkheirandish *et al.*, 2015). Homoelogeous *Btr1* and *Btr2* genes were also found in *T. dicoccoides*, *T. monococcum* and *T. durum*, located on the short arm of chromosome 3 (Avni *et al.*, 2017; Pourkheirandish *et al.*, 2018) (Tab. 1).

Non brittleness in *T. monococcum* and *T. durum* was derived by a loss of function mutation in *Btr1*. For the former, this was achieved by an amino acid substitution from alanine to threonine (Pourkheirandish *et al.*, 2018). In *T. durum* the non-shattering phenotype occurred by a 2 bp deletion in *Btr1-A* causing a frameshift mutation and a 4 kbp insertion 50 bp upstream of the stop codon of *Btr1-B*, both leading to a loss of function (Avni *et al.*, 2017). Hence targeting of *Btr1* over *Btr2* should be prioritized in a modification for a *de novo* domestication. For the GGAA wheats, genetic analysis of the *Brittle rachis* gene identified different haplotypes for Btr1-A, but amplification for Btr1-G failed. However, one identified haplotype in Btr1-A was characterized by a frameshift mutation causing changes in seven amino acids and thus proposed to alter protein function. This potential change in protein function was suggested to contribute to the loss of shattering phenotype in domesticated *T. timopheevii* (Nave *et al.*, 2021).

Non-free threshing wheats have tough glumes that cover the grains tightly and thus hamper post-harvest processing, whereas free threshing wheats have non-toughened glumes that release the grains upon threshing (Faris, 2014). Unlike for the loss of shattering, the free threshing character in wheat is controlled by multiple genes. The *Q* gene is a transcription factor belonging to the *APTETALA2* (*AP2*) family on chromosome 5A (Faris & Gill, 2002; Simons *et al.*, 2006). A change in one amino acid via a Single Nucleotide Polymorphism (SNP) and changes in the miR172 binding site caused a gain of function from *q* to *Q* (Simons *et al.*, 2006; Zhang *et al.*, 2011). The free threshing character was conferred by a change from *q* to *Q* on chromosome 5A, whereas the *q* gene on 5B was identified as a pseudo gene that was still transcriptional active (Zhang *et al.*, 2011) (Tab. 1). Therefore, the modification of *q* to *Q* on chromosome 5A should be prioritized in a *de novo* domestication. The *Q* gene has the largest effect on the free threshing phenotype but also *tg* that contributes to the tenacious glume trait is required for a full free threshing phenotype (Sharma *et al.*, 2019). *Tg* was first identified in *Ae. tauschii*. In *T. aestivum* it is located on chromosome 2D and homoeologs were found on chromosome 2A and 2B (Simonetti *et al.*, 1999; Faris *et al.*, 2014; Sharma *et al.*, 2019).The molecular mechanism behind *Tg* still needs to be resolved. *sog* on Chromosome 2 conferred threshability in *T. monococcum sinskaje,* but its role in polyploid wheats yet needs to be determined (Sood *et al.*, 2009) (Tab. 1). In a *de novo* domestication of a tetraploid wheat the combination of $tg^{2A}tg^{2B}Q$ should to be achieved due to their additive effect in threshability (Sharma *et al.*, 2019).

The formation of short plants has been a key success factor during the green revolution. *Rht-B1b* and *Rht-D1b* (*Rht = Reduced height*) located on chromosomes 4BS and 4DS were genes causing the dwarfing phenotype in wheat by interfering in the gibberellin (GA) pathway (Peng *et al.*, 1999). Likewise, this initiated negative effects regarding seedling growth due to GA insensitivity (Rebetzke *et al.*, 2011; Würschum *et al.*, 2017). The *Rht* dwarfing gene family harbored GA-sensitive genes that reduced plant height but did not impair plant growth (Ellis *et al.*, 2004). Among these genes, *Rht5* (Chr. 3BS), *Rht12* (Chr. 3AL) and *Rht13* (Chr. 7BS) showed a height reduction (Ellis *et al.*, 2005; Daoura *et al.*, 2014) but *Rht5* and *Rht12* caused a yield reduction by a reduced grain number, spikelet number per spike and Thousand Kernel Weight (TKW) (Rebetzke *et al.*, 2012; Chen *et al.*, 2013; Daoura *et al.*, 2014; Cui *et al.*, 2022). In a *de novo* domestication, the occurrence and impact of a yield reduction caused by a modification of *Rht* genes in the wild wheats should be evaluated, since their yield was already reduced compared to domesticated landrace taxa and modern cultivars.

Table 1: Overview of selected domestication alleles and loci in *Triticum* taxa

Phenotype	Wild allele	Domesticated allele	Taxa	Locus	Reference
Loss of shattering	*Btr1*	*btr1*	*T. boeoticum,* *T. monococcum*	3AS	Pourkheirandish et al. (2018)
	Btr1	*btr1*	*T. dicoccoides,* *T. durum*	3AS, 3BS	Avni et al. (2017)
Free threshing	*q*	*Q*	*T. aestivum*	5AL	Faris and Gill 2002
	Tg	*tg*	*T. dicoccoides,* *T. dicoccon and* *T. durum*	2AS, 2BS	Faris et al. (2014) (2B), Sharma (2019) (2A)
	Sog	*sog*	*T. monococum*	2AS	Sood et al. (2009)

To improve yield in *de novo* domesticated wild wheat, genes of modern bread wheat increasing spikelet number per spike and grain number per spike were proposed (Zeibig *et al.*, 2024a). *WHEAT ORTHOLOG OF APO1* (*WAPO1*) on chromosome 7AL and *FLOWERING LOCUS T2* (*FT2*) on chromosome 3AS increased spikelet number per spike and grain number per spike. Ancient alleles of the respective genes were present in *T. urartu* and *T. dicoccoides*. The modern, spikelet and grain number per spike increasing alleles were derived from a change in amino acids (Kuzay *et al.*, 2019; Glenn *et al.*, 2022; Kuzay *et al.*, 2022). Thus introducing sequences that result in the same amino acid changes in wild *Triticum* could be a potential target to improve yield traits by resembling the alleles of *WAPO1* and *FT2* of bread wheat cultivars.

The proposed traits in Zeibig *et al.* (2024a) are in accordance with target traits of other *de novo* domestication studies (DeHaan *et al.*, 2020; Yu *et al.*, 2021). Hence in the future when *de novo* domesticating species of the same genera, the identification of key target traits, such as shattering, can be proposed without thorough phenotyping. Yet, assessing the suitability of the respective location will remain a prerequisite.

5.4 Beneficial grain quality traits in wild wheat

After a successful assessment of the suitability of wild wheats to the central European climate, further beneficial characteristics in the wild wheats were identified in order to justify a *de novo* domestication.

5.4.1 Grain quality is altered by domestication which is observable as differences in quality between wild wheats and their domesticated landrace taxa

Crop wild relatives harbor beneficial nutritional characteristics that have been used for the improvement of modern crops (Hajjar & Hodgkin, 2007; Dempewolf *et al.*, 2017). Therefore, it seems that during domestication the nutritional profile of crops changed (Fernandez *et al.*, 2021; Zeibig *et al.*, 2022). For wheat this alteration was reflected in the grain micronutrient concentration and GPC which decreased from wild to domesticated taxa (Zeibig *et al.*, 2022; Zeibig *et al.*, 2024b). For the antioxidant potential and phytate concentration this pattern was not confirmed (Zeibig *et al.*, 2024b). This leads to the assumption, that the grain quality was altered during domestication for distinct parameters. In lettuce, a comparison between wild species, traditional and commercial varieties revealed higher total ascorbic acid in the wild species (Medina-Lozano *et al.*, 2021). For the total anthocyanin content, the wild species and traditional varieties did not show a significant difference (Medina-Lozano *et al.*, 2021). This supports the observation that domestication did not alter all quality parameters. However studies that grew wild and domesticated crops under the same conditions and compared for their nutritional profile are limited (Fernandez *et al.*, 2021). Therefore distinct grain quality parameters were altered during domestication, but not per se.

5.4.2 GGAA wheats harbor untapped potential in terms of grain quality traits for wheat improvement

As part of the secondary genepool of wheat, the utilization of GGAA wheats has not been a priority. However, there is increasing interest to explore their untapped diversity (Badaeva *et al.*, 2021). Especially in the light of a *de novo* domestication where no breeding barriers have to be overcome, they could be of interest. *Triticum araraticum* and *T. zhukovskyi* were both outstanding taxa regarding the grain micronutrient concentration, estimated bioavailability and GPC. Furthermore, *T. zhukovskyi* was the only taxa with elevated TPC and Oxygen Radical Absorbance Capacity (ORAC) (Zeibig *et al.*, 2024b). *Triticum timopheevii* was not recognized for superior grain quality which might suggest a more distant relationship to *T. araraticum* and *T. zhukovskyi* (Badaeva *et al.*, 2021). *T. araraticum* along with *T. zhukovskyi* harbors an untapped potential for the improvement of grain quality, especially for grain micronutrient concentration and estimated bioavailability.

5.4.3 Selection of suitable genotypes based on their grain quality, yield and cultivation characteristics for *de novo* domestication

The wild wheats were successfully analyzed for their suitability in the central European climate and beneficial grain quality characteristics. Based on their grain quality data, target genotypes were selected (Zeibig *et al.*, 2024b). These observations were combined with their cultivation suitability and yield performance in order to prioritize genotypes (Fig. 1). *Triticum araraticum* showed a high diversity and favorable grain quality traits. Based on the estimated bioavailability and grain micronutrient concentration, four *T. araraticum* and one *T. boeoticum* genotype (Tab. 2) were proposed as potential candidates for a *de novo* domestication (Zeibig *et al.*, 2024b).

Table 2: Selected genotypes for *de novo* domestication based on grain quality traits from Zeibig et al. (2024b)

Genotype No	Taxa	Genome	Genebank Identifier	Origin
27	*T. boeoticum*	A^b	PI 427583	Turkey
91	*T. araraticum*	GA	KU-8824A	Iraq
131	*T. araraticum*	GA	PI 427357	Iraq
139	*T. araraticum*	GA	KU-8713	Iraq
203	*T. araraticum*	GA	KU-1980A	Iraq

To assess their potential as future crops further, the yield parameters and phenotypic observations from the field trial (Zeibig *et al.*, 2024a) were merged with the grain quality data (Zeibig *et al.*, 2024b). For a better comparison of the individual genotypes, the values have been standardized using the z-standardization and the mean and standard deviation of the wild biological status group for the respective parameter (Fig. 1). Values > 0 for Number of tiller per plant, TKW, Grain weight per plant, Harvest Index (HI), Grain Fe concentration, Grain Zn concentration and GPC represent a superior performance in comparison to the wild biological status group. For all other traits, value < 0 are favorable.

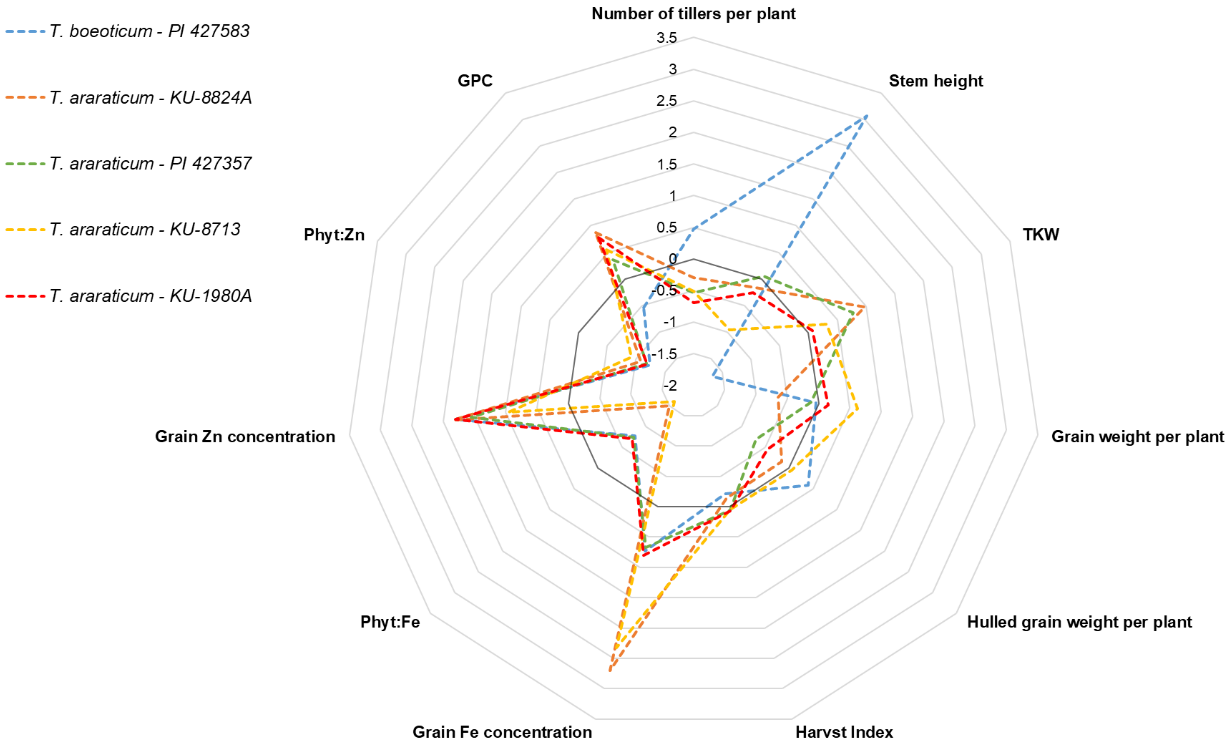

Figure 1: Comparison of cultivation, yield and grain quality traits for selected genotypes. The respective values have been standardized using z-transformation. Dotted lines represent the z-standardized trait values for each genotype in comparison to its biological status group. TKW = Thousand Kernel Weight, GPC = Grain Protein Content

The diploid *T. boeoticum* (PI 427583) showed a high grain Zn concentration and estimated bioavailability. However, for cultivation its stem height was exceptionally high compared to the average height of the wild wheats, indicating a high biomass and proneness to lodging. Further, its yield performance was not outstanding. Therefore, *T. boeoticum* PI 427583 would be ranked at the lower end of the order for a *de novo* domestication. Two *T. araraticum* genotypes (KU-8824A and KU-8713) were similar for favorable grain quality, especially for grain Fe and Zn concentration and estimated bioavailability. KU-8713 showed slightly higher grain weight per plant and a lower stem height. For *T. araraticum* KU-8824A, the TKW was higher but therefore the grain weight per plant was lowest in comparison. PI 427357 had a high TKW and low hulled grain weight, with grouping at the average grain weight per plant (Fig. 1). Grain quality traits for KU-1980A and PI 427357 were nearly similar. There were no outstanding positive or negative characteristics for cultivation and yield parameters, respectively (Fig. 1). KU-8824A and KU-8713 would be favorites for a *de novo* domestication due to better grain quality, but the other two *T. araraticum* genotypes should still be considered and evaluated, since there are more criteria and demands that need to be met for a *de novo* domestication (Chapter 5.5)

5.5 Next steps for *de novo* domestication of *T. araraticum*

The phenotypic evaluation and screening for suitable material based on the agronomic performance, suitability for target environment and beneficial grain quality traits was performed first. Genotypes of the wild GGAA wheat *T. araraticum* were identified as most promising. Following the example of wild allotetraploid rice *O. alta* (Yu *et al.*, 2021), *T. araraticum* genotypes should be evaluated regarding their transformation and regeneration efficiency rate followed by a design of a reference genome for the best performing genotype (Fig. 2).

5.5.1 Next steps – Target gene sequences

For the identification of the domestication gene sequences and subsequent design of single guide RNA (sgRNA) for genome editing, genomic information is required. Knowledge of genes and their function can be transferred between related species (Bartlett *et al.*, 2023). Since *T. araraticum* was derived from a hybridization of *T. urartu* and a relative of *Ae. speltoides*, their genome sequences (Ling *et al.*, 2013; Ling *et al.*, 2018; Avni *et al.*, 2022; Li *et al.*, 2022) could also be used as templates for gene sequences and for assembly. Also, the wild Emmer reference genome (Avni *et al.*, 2017) could serve as a source for this information. Zeng *et al.* (2020) identified putative sequences of *Btr1* and *Btr2* from *Hordeum vulgare* ssp. *spontaneum* in the *Triticeae* tribe, including *T. boeoticum*, *T. urartu*, *T. dicoccoides* and *Ae. speltoides*. These sequences can be used as a template for an identification of orthologues for the brittle rachis trait in *T. araraticum* (Tab. 3) and thus be a first step for the modification into a domesticated crop. However, some gene sequences were derived from the B genome (Tab. 3) and thus their effect need to be validated for the G genome background. Due to a common ancestry between *T. urartu*, *T. dicoccoides* and *T. araraticum* genes located on the A-genome could be more straightforward to be identified in *T. araraticum*.

Table 3: Putative *Btr1* and *Btr2* sequences in *Triticum* and *Aegilops* taxa identified in Zeng *et al.* (2020)

Taxa	Clade predicted in Zeng *et al.* (2020)	Origin/database	Chromosome	Contig or gene ID	Start-end (bp)	Nucleotide length (bp)	Reference in Zeng *et al*. (2020)
T. boeoticum	*Btr1*	NCBI	Unknown	MG596317.1	734-1324	591	Pourkheirandish *et al.* 2018
T. urartu	*Btr1*	NCBI	3A	CM009795.1	62106054-62106644	591	Ling *et al.* 2018
T. dicoccoides	*Btr1*	WEWseq v.1.0	3A		61639327-61639917	591	Avni *et al.* 2017
T. dicoccoides	*Btr1*	WEWseq v.1.0	3B		97628063-97628653	591	Avni *et al.* 2017
T. dicoccoides	*Btr1*	WEWseq v.1.0	3B		97632952-97633542	591	Avni *et al.* 2017
Ae. speltoides	*Btr1*	Assaf Distelfeld, Tel Aviv University	Unknown	scaffold_1014	602503-603093	591	Distelfeld et al. (unpublished)
T. boeoticum	*Btr2*	NCBI	Unknown	MG596329.1	389-985	597	Pourkheirandish *et al.* 2018
T. urartu	*Btr2*	NCBI	3A	CM009795.1	62078935-62078339	597	Ling *et al.* 2018
T. dicoccoides	*Btr2*	WEWseq v.1.0	3A		61601557-61600961	597	Avni *et al.* 2017
T. dicoccoides	*Btr2*	WEWseq v.1.0	3B		93998410-93997811	600	Avni *et al.* 2017
T. dicoccoides	*Btr2*	WEWseq v.1.0	3B		96349020-96348424	597	Avni *et al.* 2017
T. dicoccoides	*Btr2*	WEWseq v.1.0	3B		96864571-96863975	597	Avni *et al.* 2017
Ae. speltoides	*Btr2*	Assaf Distelfeld, Tel Aviv University	Unknown	scaffold_5549	109653-109057	597	Distelfeld et al. (unpublished)

bp = base pair

5.5.2 Next steps – Extended assessment of phenotypic data

The phenotyping and evaluation of growth, yield and grain quality has only been tested in one location for one growing season. For a more robust data regarding the cultivation and grain quality traits, the four *T. araraticum* genotypes should be grown in multiple locations (Fig. 2). This will further consider the environmental effect. Likewise, this will result in more grain material for a broader and more in depth analysis of the grain quality, like gluten content and baking quality as reviewed in Chapter 2 and in Nocente *et al.*, (2022). These results will help to determine the end use quality and suitable product development for *de novo* domesticated *T. araraticum* (Fig. 2).

5.5.3 Next steps - Performing *de novo* domestication for *T. araraticum*

After the successful implementation of a transformation protocol and the identification of the sequence and prioritization of target genes, the actual *de novo* domestication can be conducted. Recent *de novo* domestication studies choose a stacking of genes, where multiple target genes were modified at once (Lemmon *et al.*, 2018; Zsögön *et al.*, 2018). The advantage of this approach was a shorter time duration leading to a *de novo* domesticated crop. Yet this approach need to be treated with caution when it comes to the modification of pleiotropic genes (Schaart *et al.*, 2021). Furthermore, a good understanding of the gene's role in the plant homeostasis is required (Bartlett *et al.*, 2023). Phenotypic assessment of the transformed plants is necessary because of the possibility of multiple genes contributing to one trait. Another challenge is the tetraploidy of *T. araraticum*, because all gene copies on the sub-genomes need to be edited due to functional redundancy (Kim *et al.*, 2018). Therefore, similar conserved regions in the homologous sequences of the sub-genomes can be identified and targeted by one sgRNA during genome editing. In case of no such regions, individual sgRNAs for each gene copy need to be designed (Zhou *et al.*, 2023) (Fig. 2).

The obstacles for a *de novo* domestication of *T. araraticum* can be overcomed using genomic and molecular biology methods. Yet, suitable future use scenarios for cultivating *de novo* domesticated wild wheat need to be developed.

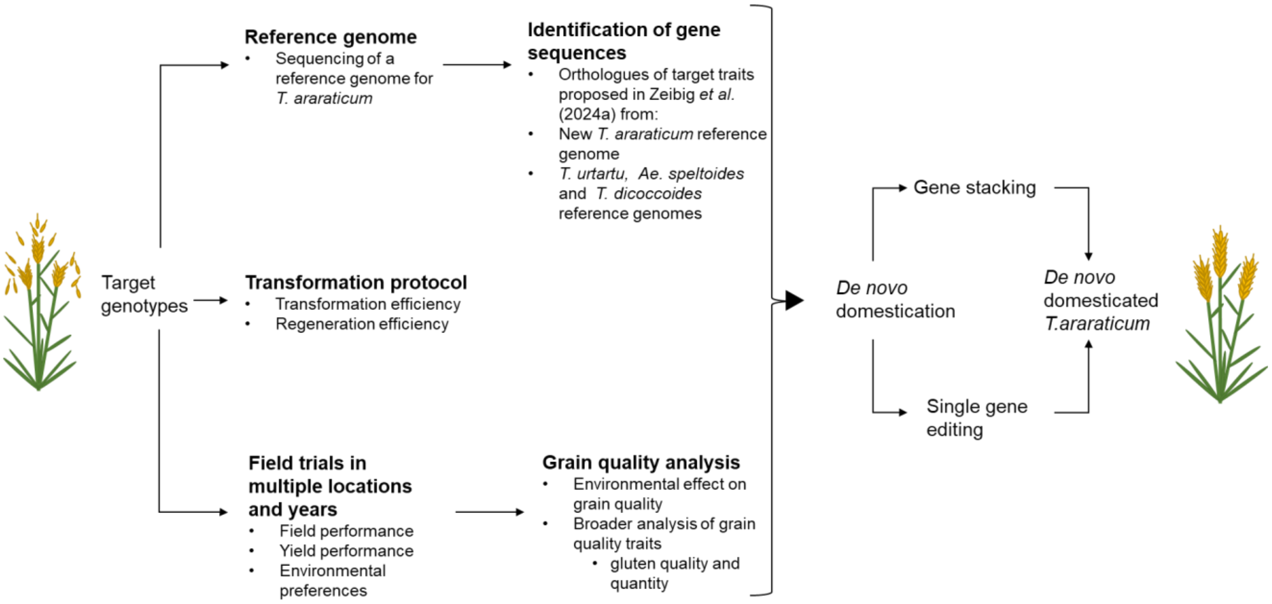

Figure 2: Schematic overview for proposed next steps for a *de novo* domestication of *T. araraticum*

5.6 Future scenarios for *de novo* domesticated *T. araraticum*

Performing *de novo* domestication in wild wheat is a time consuming and work intensive task. In order to justify this amount of work and to contribute to food security, future use scenarios for *de novo* domesticated wild wheat will be outlined.

5.6.1 Scenario 1 - *De novo* domesticated *T. araraticum* in a modern wheat cultivation system

The most obvious scenario is to grow *de novo* domesticated wild wheat with elevated grain quality as a single crop in the field, following the example of modern wheat. This can broaden the crop rotation and thus diversity in the field. *De novo* domesticated *T. araraticum* would be grown like modern wheat cultivars including soil preparation, sowing practice and adjusted external inputs (Fig. 3). First of all, optimal sowing depth and density need to be identified. For wheat, the ideal sowing depth is 2-4 cm and sowing density is 200-400 seeds/m² depending on the sowing date. For *T. araraticum*, germination rate was observed to be lower (Zeibig, unpublished data/personal observation) and wild seeds have a high dormancy (Meyer & Purugganan, 2013). Studies on wild wheat germination rate are not existent and in Zeibig *et al.* (2024a) plants were pre-cultivated in a greenhouse before transplanted to the field. Therefore, higher sowing density would be suggested to ensure plant density. A high plant density is favorable for the suppression of weeds (Weiner *et al.*, 2001) but increases intraspecific competition for nutrients, water and light vice versa. Modern wheats exhibit an erectophil phenotype, thus maximizing radiation use efficiency (RUE) for a higher photosynthetic rate in dense stands (Reynolds *et al.*, 2009). *Triticum araraticum* showed a broader canopy and therefore shading of leaves could occur, which hampers photosynthesis. Yield potential is the product of light intercepted, RUE and HI (Reynolds *et al.*, 2012). Currently, the HI of *T. araraticum* was low and as elaborated earlier, its RUE might not be ideal. In conclusion, the current yield potential of a *de novo* domesticated *T. araraticum* is estimated to be low. This is further supported by the yield inferiority of wild wheats compared to landraces and modern cultivars (Zeibig *et al.*, 2024a). Yield has been a selection target from the onset of domestication and still continues to date as it ensure food security and economic revenue for farmers (Law *et al.*, 1978; Reynolds *et al.*, 2012; Hawkesford *et al.*, 2013; Meyer & Purugganan, 2013). Yield is a quantitative genetic trait and is comprised of different components, such as TKW, grain number per spike, spikelets per spike and spike number (Cao *et al.*, 2020). Genes for yield increase by the modification of the spikelets per spike and grain number were proposed in Zeibig *et al.* (2024a). However, due to the quantitative genetic base and the long breeding efforts that have been invested in yield it will be difficult to achieve a similar yield level in *T. araraticum* like in modern wheat cultivars solely by genetic modification. Therefore, after a modification of the key domestication genes, a subsequent breeding program for yield improvement should be established without compromising on the beneficial grain quality traits.

Instead of treating *de novo* domesticated wheat like a modern wheat cultivar applying identical expectations to it, taking advantages of its unique characteristics might result in a more suitable scenario. Arable land degradation imposes a huge threat to food security. Degradation processes involve aridity, soil erosion, salinization and loss of soil organ carbon that will challenge crop production on these soils (Prăvălie *et al.*, 2021). Wild Emmer wheat and *T. urartu* showed a good adaptation potential to drought stress (Peleg *et al.*, 2005; Pour-Aboughadareh *et al.*, 2017) and *T. boeoticum* increased antioxidants to cope with salinity (Ahmadi *et al.*, 2018). For *T. araraticum* no such studies are existent to date. In areas where modern wheat cannot grow due to high presence of abiotic stresses and soil degradation, *de novo* domesticated wheats which can thrive in these harsh conditions can be cultivated and thus maintain a share of yield and productivity.

In conclusion, growing *de novo* domesticated *T. araraticum* like modern wheat cultivars in their respective environment will have a low chance of realization due to yield inferiority. However, in fields where modern wheat cultivars might fail due to environmental constraints, they could inhabit that niche and maintain productivity (Fig. 3).

5.6.2 Scenario 2 – *De novo* domesticated *T. araraticum* in a wheat variety mixture to increase wheat flour quality

Growing a mix of varieties of the same species is a strategy to maintain yield stability while increasing diversity on the field and reducing external inputs (Kiær *et al.*, 2009). Different varieties increased yield and yield stability by compensating for biotic and abiotic stresses, mitigating diseases and spread, and utilizing resources differently (Kiær *et al.*, 2009; Wuest *et al.*, 2021; Vestergaard & Jørgensen, 2024). Bread and durum wheat were both cultivated successfully in a cultivar mixture and higher yields compared to monoculture systems were observed while maintaining GPC (Döring *et al.*, 2015; Montazeaud *et al.*, 2020). Adding *de novo* domesticated wheat to a cultivar mixture with modern wheat cultivars can be a good option to increase the micronutrient content of the flour while sustaining a high yield at the same time (Fig. 3). Due to a synchronized phenology, the *de novo* domesticated *T. araraticum* aligns with the modern wheat cultivars (Zeibig *et al.*, 2024a). Furthermore, lodging was observed to be reduced in mixtures (Vestergaard & Jørgensen, 2024), which would be favorable for *de novo* domesticated *T. araraticum*. Since it has a wild growth habit and was not designed for a monocrop system, it could also thrive in a mixed cropping system and capture different niches not utilized by the modern cultivars (Wuest *et al.*, 2021). Therefore the application of external inputs could be reduced (Wuest *et al.*, 2021; Vestergaard & Jørgensen, 2024). By combining modern wheat cultivars and *de novo* domesticated *T. araraticum* the lower yield potential of *de novo* domesticated *T. araraticum* would be compensated and its elevated grain quality could improve the nutritional value.

5.6.3 Scenario 3 – *De novo* domesticated wild wheat as a pre-breeding resource

Wild wheats offer favorable traits and have been recognized as a cradle of genetic and trait diversity. To ensure genetic gain of wheat, landraces and wild wheats have been recognized as a great source to introduce diversity (Kilian *et al.*, 2021; Sukumaran *et al.*, 2022). Main issues when crossing elite cultivars with landraces or wild wheats are crossing incompatibility, infertility and linkage drag (Sharma *et al.*, 2013; Bohra *et al.*, 2022; Sukumaran *et al.*, 2022). The latter process will incorporate unfavorable traits that hamper the performance of modern cultivars. Especially wild traits are unwanted and to eliminate them, backcrosses or elite crosses are needed which is a time consuming process (Sharma *et al.*, 2021; Sukumaran *et al.*, 2022). Therefore, landraces are preferred for crosses with modern cultivars to incorporate new traits and wild wheats are neglected in pre-breeding programs (Sharma *et al.*, 2021). By eliminating characteristic wild traits such as grain shattering and thus creating *de novo* domesticated wheats that equal landraces, wild wheat’s potential use as pre-breeding material could be increased (Fig. 3). However, crosses of *de novo* domesticated *T. araraticum* and modern wheat cultivars will still be challenging due to different chromosome number and origin (Tadesse *et al.*, 2019; King *et al.*, 2022). Furthermore, the target traits need to be defined for the respective pre-breeding program and whether they are of qualitative or quantitative nature. The former is easier to integrate in modern cultivars and its successful incorporation can be determined by genetic markers (Sukumaran *et al.*, 2022). For quantitative traits, this is more challenging due to the large genotype x environment effect and cultivar-specific expression

(Sharma *et al.*, 2021; Sukumaran *et al.*, 2022). Still the use of *de novo* domesticated wild wheat would broaden the access to plant genetic resource. Its unique characteristics would be used indirectly by introducing them in the genetic background of elite cultivars (Sukumaran *et al.*, 2022).

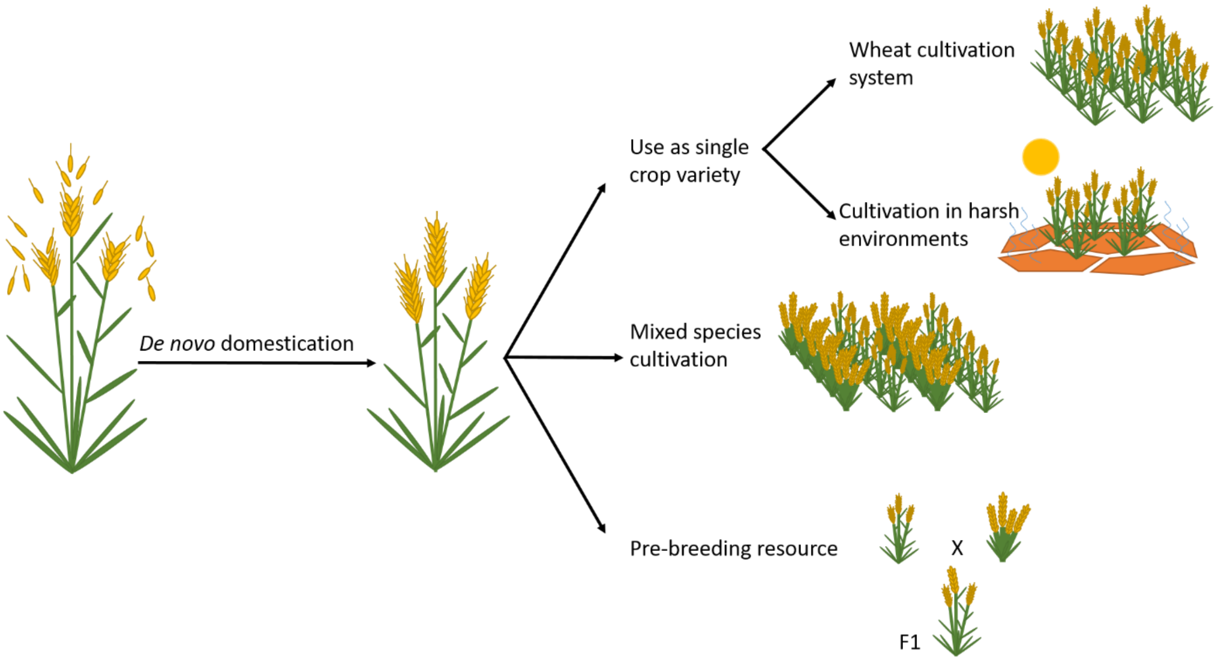

Figure 3: Possible future cultivation scenarios for *de novo* domesticated *T. araraticum*

Three different scenarios for the potential use of *de novo* domesticated *T. araraticum* have been outlined. Using them in species mixtures or as pre-breeding resource seems to be more suitable than as a single crop. Eventually, the application will also depend on consumer acceptance as well as the regulatory framework in the respective country. From a scientific point of view, *de novo* domesticated *T. araraticum* could be one component in building a more diverse and sustainable cropping system with providing nutritious food.

6 Conclusion

Wheat wild relatives harbor a broad diversity which confers advantageous traits for biotic and abiotic stresses as well as for nutritional value. With the advent of genome editing, *de novo* domestication of wild wheats is a new approach, which directly modifies the wild wheats into cultivable crops instead of using them as a gene donor. In this thesis, the feasibility of wild wheats for a *de novo* domestication was assessed. Therefore, the suitability of the wild wheats for the target environment as well as target traits were analyzed. Further, genotypes with outstanding grain quality characteristics were identified and selected.

The wild wheats showed a good suitability to the central European climate. Wild traits such as grain shattering clearly distinguished the wild wheats from the landrace taxa and modern wheat cultivars. Other traits were tightly hulled glumes, plant height and yield and target genes for modification of these traits for a *de novo* domestication were proposed. Grain quality traits were altered during domestication. The grain quality analysis revealed outstanding grain micronutrient concentration and estimated bioavailability, GPC and antioxidant potential within the GGAA wheats. Among the wild wheats, especially *T. araraticum* showed excellent characteristics for the respective traits except for the antioxidant potential, which was only superior in *T. zhukovskyi.* Therefore, four *T. araraticum* genotypes were selected as target genotypes for a *de novo* domestication.

Future steps for a *de novo* domestication would now comprise further phenotyping in the field at different locations and analysis of grain quality characteristics, along with establishing a transformation protocol and a reference genome as well as the identification of orthologues. Furthermore, future use scenarios for *de novo* domesticated *T. araraticum* were outlined, indicating that also the cultivation system needs to be suitable for the crop’s requirement and that it should not be transferred to a modern wheat cropping system directly. Hence, *de novo* domesticated wheats can complement the cultivation system and increase diversity, but includes laborious tasks to reach this goal.

7 References

Ahmadi J, Pour-Aboughadareh A, Fabriki-Ourang S, Mehrabi A-A, Siddique KHM. 2018. Screening wild progenitors of wheat for salinity stress at early stages of plant growth: insight into potential sources of variability for salinity adaptation in wheat. *Crop and Pasture Science* **69**: 649.

Ahmar S, Hensel G, Gruszka D. 2023. CRISPR/Cas9-mediated genome editing techniques and new breeding strategies in cereals – current status, improvements, and perspectives. *Biotechnology advances* **69**: 108248.

Ali MW, Borrill P. 2020. Applying genomic resources to accelerate wheat biofortification. *Heredity* **125**: 386–395.

Arora S, Cheema J, Poland J, Uauy C, Chhuneja P. 2019. Genome-Wide Association Mapping of Grain Micronutrients Concentration in Aegilops tauschii. *Frontiers in Plant Science* **10**: 54.

Avni R, Lux T, Minz-Dub A, Millet E, Sela H, Distelfeld A, Deek J, Yu G, Steuernagel B, Pozniak C *et al.* 2022. Genome sequences of three Aegilops species of the section Sitopsis reveal phylogenetic relationships and provide resources for wheat improvement. *The Plant Journal* **110**: 179–192.

Avni R, Nave M, Barad O, Baruch K, Twardziok SO, Gundlach H, Hale I, Mascher M, Spannagl M, Wiebe K *et al.* 2017. Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. *Science (New York, N.Y.)* **357**: 93–97.

Avni R, Zhao R, Pearce S, Jun Y, Uauy C, Tabbita F, Fahima T, Slade A, Dubcovsky J, Distelfeld A. 2014. Functional characterization of GPC-1 genes in hexaploid wheat. *Planta* **239**: 313–324.

Badaeva ED, Konovalov FA, Knüpffer H, Fricano A, Ruban AS, Kehel Z, Zoshchuk SA, Surzhikov SA, Neumann K, Graner A *et al.* 2021. Genetic diversity, distribution and domestication history of the neglected GGA t A t genepool of wheat. doi: 10.1101/2021.01.10.426084.

Bartlett ME, Moyers BT, Man J, Subramaniam B, Makunga NP. 2023. The Power and Perils of De Novo Domestication Using Genome Editing. *Annual review of plant biology* **74**: 727–750.

Biel W, Jaroszewska A, Stankowski S, Sobolewska M, Kępińska-Pacelik J. 2021. Comparison of yield, chemical composition and farinograph properties of common and ancient wheat grains. *European Food Research and Technology*. doi: 10.1007/s00217-021-03729-7.

Biesiekierski JR. 2017. What is gluten? *Journal of gastroenterology and hepatology* **32 Suppl 1**: 78–81.

Bohra A, Kilian B, Sivasankar S, Caccamo M, Mba C, McCouch SR, Varshney RK. 2022. Reap the crop wild relatives for breeding future crops. *Trends in Biotechnology* **40**: 412–431.

Brozynska M, Furtado A, Henry RJ. 2016. Genomics of crop wild relatives: expanding the gene pool for crop improvement. *Plant biotechnology journal* **14**: 1070–1085.

Cakmak I, Ozkan H, Braun HJ, Welch RM, Romheld V. 2000. Zinc and Iron Concentrations in Seeds of Wild, Primitive, and Modern Wheats. *Food and Nutrition Bulletin* **21**: 401–403.

Cakmak I, Pfeiffer WH, McClafferty B. 2010. REVIEW: Biofortification of Durum Wheat with Zinc and Iron. *Cereal Chemistry Journal* **87**: 10–20.

Cakmak I, Torun A, Millet E, Feldman M, Fahima T, Korol A, Nevo E, Braun HJ, Özkan H. 2004. Triticum dicoccoides : An important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. *Soil Science and Plant Nutrition* **50**: 1047–1054.

Cao S, Xu D, Hanif M, Xia X, He Z. 2020. Genetic architecture underpinning yield component traits in wheat. *Theoretical and Applied Genetics* **133**: 1811–1823.

Capdeville N, Schindele P, Puchta H. 2023. Getting better all the time — recent progress in the development of CRISPR/Cas-based tools for plant genome engineering. *Current opinion in biotechnology* **79**: 102854.

Cardi T, Murovec J, Bakhsh A, Boniecka J, Bruegmann T, Bull SE, Eeckhaut T, Fladung M, Galovic V, Linkiewicz A *et al.* 2023. CRISPR/Cas-mediated plant genome editing: outstanding challenges a decade after implementation. *Trends in Plant Science* **28**: 1144–1165.

Chatzav M, Peleg Z, Ozturk L, Yazici A, Fahima T, Cakmak I, Saranga Y. 2010. Genetic diversity for grain nutrients in wild emmer wheat: potential for wheat improvement. *Annals of botany* **105**: 1211–1220.

Chen K, Wang Y, Zhang R, Zhang H, Gao C. 2019. CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture. *Annual review of plant biology* **70**: 667–697.

Chen L, Phillips AL, Condon AG, Parry MAJ, Hu Y-G. 2013. GA-responsive dwarfing gene Rht12 affects the developmental and agronomic traits in common bread wheat. *PloS one* **8**: e62285.

Chhuneja P, Dhaliwal HS, Bains NS, Singh K. 2006. Aegilops kotschyi and Aegilops tauschii as sources for higher levels of grain Iron and Zinc. *Plant Breeding* **125**: 529–531.

Crespo-Herrera LA, Crossa J, Vargas M, Braun H-J. 2022. Defining Target Wheat Breeding Environments. In: Reynolds MP, Braun H-J, eds. *Wheat Improvement*. Cham: Springer International Publishing, 31–45.

Cui C, Lu Q, Zhao Z, Lu S, Duan S, Yang Y, Qiao Y, Chen L, Hu Y-G. 2022. The fine mapping of dwarf gene Rht5 in bread wheat and its effects on plant height and main agronomic traits. *Planta* **255**: 114.

Curtin S, Qi Y, Peres LEP, Fernie AR, Zsögön A. 2021. Pathways to de novo domestication of crop wild relatives. *PLANT PHYSIOLOGY*. doi: 10.1093/plphys/kiab554.

Daoura BG, Chen L, Du Y, Hu Y-G. 2014. Genetic effects of dwarfing gene Rht-5 on agronomic traits in common wheat (Triticum aestivum L.) and QTL analysis on its linked traits. *Field Crops Research* **156**: 22–29.

Debnath S, Dey A, Khanam R, Saha S, Sarkar D, Saha JK, Coumar MV, Patra BC, Biswas T, Ray M *et al.* 2023. Historical shifting in grain mineral density of landmark rice and wheat cultivars released over the past 50 years in India. *Scientific reports* **13**: 21164.

DeHaan L, Larson S, López-Marqués RL, Wenkel S, Gao C, Palmgren M. 2020. Roadmap for Accelerated Domestication of an Emerging Perennial Grain Crop. *Trends in Plant Science* **25**: 525–537.

Dempewolf H, Baute G, Anderson J, Kilian B, Smith C, Guarino L. 2017. Past and Future Use of Wild Relatives in Crop Breeding. *Crop Science* **57**: 1070–1082.

Distelfeld A, Cakmak I, Peleg Z, Ozturk L, Yazici AM, Budak H, Saranga Y, Fahima T. 2007. Multiple QTL-effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations. *Physiologia Plantarum* **129**: 635–643.

Doebley JF, Gaut BS, Smith BD. 2006. The molecular genetics of crop domestication. *Cell* **127**: 1309–1321.

Döring TF, Annicchiarico P, Clarke S, Haigh Z, Jones HE, Pearce H, Snape J, Zhan J, Wolfe MS. 2015. Comparative analysis of performance and stability among composite cross populations, variety mixtures and pure lines of winter wheat in organic and conventional cropping systems. *Field Crops Research* **183**: 235–245.

Ellis MH, Rebetzke GJ, Azanza F, Richards RA, Spielmeyer W. 2005. Molecular mapping of gibberellin-responsive dwarfing genes in bread wheat. *Theoretical and Applied Genetics* **111**: 423–430.

Ellis MH, Rebetzke GJ, Chandler P, Bonnett D, Spielmeyer W, Richards RA. 2004. The effect of different height reducing genes on the early growth of wheat. *Functional plant biology : FPB* **31**: 583–589.

Erenstein O, Jaleta M, Mottaleb KA, Sonder K, Donovan J, Braun H-J. 2022. Global Trends in Wheat Production, Consumption and Trade. In: Reynolds MP, Braun H-J, eds. *Wheat Improvement*. Cham: Springer International Publishing, 47–66.

Faris JD. 2014. Wheat Domestication: Key to Agricultural Revolutions Past and Future. In: Tuberosa R, Graner A, Frison E, eds. *Genomics of Plant Genetic Resources*. Dordrecht: Springer Netherlands, 439–464.

Faris JD, Gill BS. 2002. Genomic targeting and high-resolution mapping of the domestication gene Q in wheat. *Genome* **45**: 706–718.

Faris JD, Zhang Z, Chao S. 2014. Map-based analysis of the tenacious glume gene Tg-B1 of wild emmer and its role in wheat domestication. *Gene* **542**: 198–208.

Feldman M, Levy AA. 2023. Wheat Evolution and Domestication. Cham: Springer International Publishing.

Fernandez AR, Sáez A, Quintero C, Gleiser G, Aizen MA. 2021. Intentional and unintentional selection during plant domestication: herbivore damage, plant defensive traits and nutritional quality of fruit and seed crops. *The New phytologist* **231**: 1586–1598.

Fernie AR, Alseekh S, Liu J, Yan J. 2021. Using Precision Phenotyping to Inform de novo Domestication. *PLANT PHYSIOLOGY*. doi: 10.1093/plphys/kiab160.

Fernie AR, Yan J. 2019. De Novo Domestication: An Alternative Route toward New Crops for the Future. *Molecular plant* **12**: 615–631.

Foulkes MJ, Molero G, Griffiths S, Slafer GA, Reynolds MP. 2022. Yield Potential. In: Reynolds MP, Braun H-J, eds. *Wheat Improvement*. Cham: Springer International Publishing, 379–396.

Gasparini K, Moreira JDR, Peres LEP, Zsögön A. 2021. De novo domestication of wild species to create crops with increased resilience and nutritional value. *Current opinion in plant biology* **60**: 102006.

Glenn P, Zhang J, Brown-Guedira G, DeWitt N, Cook JP, Li K, Akhunov E, Dubcovsky J. 2022. Identification and characterization of a natural polymorphism in FT-A2 associated with increased number of grains per spike in wheat. *Theoretical and Applied Genetics* **135**: 679–692.

Gómez-Fernández A, Aranda I, Milla R. 2024. Early human selection of crops' wild progenitors explains the acquisitive physiology of modern cultivars. *Nature plants*. doi: 10.1038/s41477-023-01588-6.

Govindan V, Michaux KD, Pfeiffer WH. 2022. Nutritionally Enhanced Wheat for Food and Nutrition Security. In: Reynolds MP, Braun H-J, eds. *Wheat Improvement*. Cham: Springer International Publishing, 195–214.

Gross BL, Olsen KM. 2010. Genetic perspectives on crop domestication. *Trends in Plant Science* **15**: 529–537.

Gupta PK, Balyan HS, Sharma S, Kumar R. 2020. Biofortification and bioavailability of Zn, Fe and Se in wheat: present status and future prospects. *Theoretical and Applied Genetics*. doi: 10.1007/s00122-020-03709-7.

Guzmán C, Ibba MI, Álvarez JB, Sissons M, Morris C. 2022. Wheat Quality. In: Reynolds MP, Braun H-J, eds. *Wheat Improvement*. Cham: Springer International Publishing, 177–193.

Hajjar R, Hodgkin T. 2007. The use of wild relatives in crop improvement: a survey of developments over the last 20 years. *Euphytica* **156**: 1–13.

Hancock JF. 2005. Contributions of domesticated plant studies to our understanding of plant evolution. *Annals of botany* **96**: 953–963.

Hawkesford MJ, Araus J-L, Park R, Calderini D, Miralles D, Shen T, Zhang J, Parry MAJ. 2013. Prospects of doubling global wheat yields. *Food and energy security* **2**: 34–48.

Hebelstrup KH, Azariadis A, Cordes A, Henriksen PS, Brinch-Pedersen H. 2023. Prehistoric Plant Exploitation and Domestication: An Inspiration for the Science of De Novo Domestication in Present Times. *Plants (Basel, Switzerland)* **12**.

Igrejas G, Ikeda TM, Guzmán C, eds. 2020. Wheat quality for improving processing and human health. Cham, Switzerland: Springer.

Jaradat AA. 2017. Agriculture in the Fertile Crescent: continuity and change under climate change. *CABI Reviews* **2017**: 1–33.

Jian L, Yan J, Liu J. 2022. De Novo Domestication in the Multi-Omics Era. *Plant & cell physiology* **63**: 1592–1606.

Johns T, Eyzaguirre PB. 2007. Biofortification, biodiversity and diet: A search for complementary applications against poverty and malnutrition. *Food Policy* **32**: 1–24.

Kantar MB, Nashoba AR, Anderson JE, Blackman BK, Rieseberg LH. 2017. The Genetics and Genomics of Plant Domestication. *BioScience* **67**: 971–982.

Khan MZ, Zaidi SS-E-A, Amin I, Mansoor S. 2019. A CRISPR Way for Fast-Forward Crop Domestication. *Trends in Plant Science* **24**: 293–296.

Kiær LP, Skovgaard IM, Østergård H. 2009. Grain yield increase in cereal variety mixtures: A meta-analysis of field trials. *Field Crops Research* **114**: 361–373.

Kilian B, Dempewolf H, Guarino L, Werner P, Coyne C, Warburton ML. 2021. Crop Science special issue: Adapting agriculture to climate change: A walk on the wild side. *Crop Science* **61**: 32–36.

Kim D, Alptekin B, Budak H. 2018. CRISPR/Cas9 genome editing in wheat. *Functional & integrative genomics* **18**: 31–41.

King J, Grewal S, Fellers JP, King IP. 2022. Exploring Untapped Wheat Genetic Resources to Boost Food Security. In: Reynolds MP, Braun H-J, eds. *Wheat Improvement*. Cham: Springer International Publishing, 319–340.

Kuzay S, Lin H, Li C, Chen S, Woods DP, Zhang J, Lan T, Korff M von, **Dubcovsky J. 2022.** WAPO-A1 is the causal gene of the 7AL QTL for spikelet number per spike in wheat. *PLoS genetics* **18**: e1009747.

Kuzay S, Xu Y, Zhang J, Katz A, Pearce S, Su Z, Fraser M, Anderson JA, Brown-Guedira G, DeWitt N *et al.* 2019. Identification of a candidate gene for a QTL for spikelet number per spike on wheat chromosome arm 7AL by high-resolution genetic mapping. *Theoretical and Applied Genetics* **132**: 2689–2705.

Ladizinsky G. 1985. Founder effect in crop-plant evolution. *Economic Botany* **39**: 191–199.

Lafiandra D, Riccardi G, Shewry PR. 2014. Improving cereal grain carbohydrates for diet and health. *Journal of Cereal Science* **59**: 312–326.

Langridge P, Alaux M, Almeida NF, Ammar K, Baum M, Bekkaoui F, Bentley AR, Beres BL, Berger B, Braun H-J *et al.* 2022. Meeting the Challenges Facing Wheat Production: The Strategic Research Agenda of the Global Wheat Initiative. *Agronomy* **12**: 2767.

Law CN, Snape JW, Worland AJ. 1978. The genetical relationship between height and yield in wheat. *Heredity* **40**: 133–151.

Lemmon ZH, Reem NT, Dalrymple J, Soyk S, Swartwood KE, Rodriguez-Leal D, van Eck J, Lippman ZB. 2018. Rapid improvement of domestication traits in an orphan crop by genome editing. *Nature plants* **4**: 766–770.

Li L-F, Zhang Z-B, Wang Z-H, Li N, Sha Y, Wang X-F, Ding N, Li Y, Zhao J, Wu Y *et al.* 2022. Genome sequences of five Sitopsis species of Aegilops and the origin of polyploid wheat B subgenome. *Molecular plant*. doi: 10.1016/j.molp.2021.12.019.

Li T, Yang X, Yu Y, Si X, Zhai X, Zhang H, Dong W, Gao C, Xu C. 2018. Domestication of wild tomato is accelerated by genome editing. *Nature biotechnology*. doi: 10.1038/nbt.4273.

Ling H-Q, Ma B, Shi X, Liu H, Dong L, Sun H, Cao Y, Gao Q, Zheng S, Li Y *et al.* 2018. Genome sequence of the progenitor of wheat A subgenome Triticum urartu. *Nature* **557**: 424–428.

Ling H-Q, Zhao S, Liu D, Wang J, Sun H, Zhang C, Fan H, Li D, Dong L, Tao Y *et al.* 2013. Draft genome of the wheat A-genome progenitor Triticum urartu. *Nature* **496**: 87–90.

Luo G, Najafi J, Correia PMP, Trinh MDL, Chapman EA, Østerberg JT, Thomsen HC, Pedas PR, Larson S, Gao C *et al.* 2022. Accelerated Domestication of New Crops: Yield is Key. *Plant & cell physiology*. doi: 10.1093/pcp/pcac065.

Medina-Lozano I, Bertolín JR, Díaz A. 2021. Nutritional value of commercial and traditional lettuce (Lactuca sativa L.) and wild relatives: Vitamin C and anthocyanin content. *Food Chemistry* **359**: 129864.

Meyer RS, Purugganan MD. 2013. Evolution of crop species: genetics of domestication and diversification. *Nature reviews. Genetics* **14**: 840–852.

Milla R, Osborne CP. 2021. Crop origins explain variation in global agricultural relevance. *Nature plants* **7**: 598–607.

Monasterio I, Graham RD. 2000. Breeding for Trace Minerals in Wheat. *Food and Nutrition Bulletin* **21**: 392–396.

Montazeaud G, Violle C, Roumet P, Rocher A, Ecarnot M, Compan F, Maillet G, Fort F, Fréville H. 2020. Multifaceted functional diversity for multifaceted crop yield: Towards ecological assembly rules for varietal mixtures. *Journal of Applied Ecology* **57**: 2285–2295.

Nave M, Taş M, Raupp J, Tiwari VK, Ozkan H, Poland J, Hale I, Komatsuda T, Distelfeld A. 2021. The Independent Domestication of Timopheev's Wheat: Insights from Haplotype Analysis of the Brittle rachis 1 (BTR1-A) Gene. *Genes* **12**.

Nocente F, Galassi E, Taddei F, Natale C, Gazza L. 2022. Ancient Caucasian Wheats: A Contribution for Sustainable Diets and Food Diversity. *Foods (Basel, Switzerland)* **11**.

Østerberg JT, Xiang W, Olsen LI, Edenbrandt AK, Vedel SE, Christiansen A, Landes X, Andersen MM, Pagh P, Sandøe P *et al.* 2017. Accelerating the Domestication of New Crops: Feasibility and Approaches. *Trends in Plant Science* **22**: 373–384.

Peleg Z, Fahima T, Abbo S, Krugman T, Nevo E, Yakir D, Saranga Y. 2005. Genetic diversity for drought resistance in wild emmer wheat and its ecogeographical associations. *Plant, Cell and Environment* **28**: 176–191.

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F *et al.* 1999. 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* **400**: 256–261.

Piironen V, Salmenkallio-Marttila M. 2009. CHAPTER 7: Micronutrients and Phytochemicals in Wheat Grain. In: Khan K, Shewry PR, eds. *WHEAT: Chemistry and Technology*. 3340 Pilot Knob Road, St. Paul, Minnesota 55121, U.S.A.: AACC International, Inc, 179–222.

Poole N, Donovan J, Erenstein O. 2021. Viewpoint: Agri-nutrition research: Revisiting the contribution of maize and wheat to human nutrition and health. *Food Policy* **100**: 101976.

Pour-Aboughadareh A, Ahmadi J, Mehrabi AA, Etminan A, Moghaddam M, Siddique KHM. 2017. Physiological responses to drought stress in wild relatives of wheat: implications for wheat improvement. *Acta Physiologiae Plantarum* **39**.

Pourkheirandish M, Dai F, Sakuma S, Kanamori H, Distelfeld A, Willcox G, Kawahara T, Matsumoto T, Kilian B, Komatsuda T. 2018. On the Origin of the Non-brittle Rachis Trait of Domesticated Einkorn Wheat. *Frontiers in Plant Science* **8**: 2031.

Pourkheirandish M, Hensel G, Kilian B, Senthil N, Chen G, Sameri M, Azhaguvel P, Sakuma S, Dhanagond S, Sharma R *et al.* 2015. Evolution of the Grain Dispersal System in Barley. *Cell* **162**: 527–539.

Prăvălie R, Patriche C, Borrelli P, Panagos P, Roșca B, Dumitraşcu M, Nita I-A, Săvulescu I, Birsan M-V, Bandoc G. 2021. Arable lands under the pressure of multiple land degradation processes. A global perspective. *Environmental research* **194**: 110697.

Rawat N, Tiwari VK, Singh N, Randhawa GS, Singh K, Chhuneja P, Dhaliwal HS. 2009. Evaluation and utilization of Aegilops and wild Triticum species for enhancing iron and zinc content in wheat. *Genetic Resources and Crop Evolution* **56**: 53–64.

Razzaq A, Saleem F, Wani SH, Abdelmohsen SAM, Alyousef HA, Abdelbacki AMM, Alkallas FH, Tamam N, Elansary HO. 2021. De-novo Domestication for Improving Salt Tolerance in Crops. *Frontiers in plant science* **12**: 681367.

Rebetzke GJ, Ellis MH, Bonnett DG, Condon AG, Falk D, Richards RA. 2011. The Rht13 dwarfing gene reduces peduncle length and plant height to increase grain number and yield of wheat. *Field Crops Research* **124**: 323–331.

Rebetzke GJ, Ellis MH, Bonnett DG, Mickelson B, Condon AG, Richards RA. 2012. Height reduction and agronomic performance for selected gibberellin-responsive dwarfing genes in bread wheat (Triticum aestivum L.). *Field Crops Research* **126**: 87–96.

Reynolds M, Foulkes J, Furbank R, Griffiths S, King J, Murchie E, Parry M, Slafer G. 2012. Achieving yield gains in wheat. *Plant, Cell and Environment* **35**: 1799–1823.

Reynolds M, Foulkes MJ, Slafer GA, Berry P, Parry MAJ, Snape JW, Angus WJ. 2009. Raising yield potential in wheat. *Journal of experimental botany* **60**: 1899–1918.

Reynolds MP, Braun H-J. 2022. Wheat Improvement. In: Reynolds MP, Braun H-J, eds. *Wheat Improvement*. Cham: Springer International Publishing, 3–15.

Ross-Ibarra J, Morrell PL, Gaut BS. 2007. Plant domestication, a unique opportunity to identify the genetic basis of adaptation. *Proceedings of the National Academy of Sciences of the United States of America* **104 Suppl 1**: 8641–8648.

Salamini F, Ozkan H, Brandolini A, Schäfer-Pregl R, Martin W. 2002. Genetics and geography of wild cereal domestication in the near east. *Nature reviews. Genetics* **3**: 429–441.

Schaart JG, van de Wiel CCM, Smulders MJM. 2021. Genome editing of polyploid crops: prospects, achievements and bottlenecks. *Transgenic research* **30**: 337–351.

Schoen A, Saripalli G, Hosseinirad S, Sharma PK, Kajla A, Yadav IS, Tiwari V. 2024. Genome Sequences from Diploids and Wild Relatives of Wheat for Comparative Genomics and Alien Introgressions. In: Appels R, Eversole K, Feuillet C, Gallagher D, eds. *The Wheat Genome*. Cham: Springer International Publishing, 241–263.

Sharma JS, Running KLD, Xu SS, Zhang Q, Peters Haugrud AR, Sharma S, McClean PE, Faris JD. 2019. Genetic analysis of threshability and other spike traits in the evolution of cultivated emmer to fully domesticated durum wheat. *Molecular genetics and genomics : MGG* **294**: 757–771.

Sharma S, Schulthess AW, Bassi FM, Badaeva ED, Neumann K, Graner A, Özkan H, Werner P, Knüpffer H, Kilian B. 2021. Introducing Beneficial Alleles from Plant Genetic Resources into the Wheat Germplasm. *Biology* **10**: 982.

Sharma S, Upadhyaya HD, Varshney RK, Gowda CLL. 2013. Pre-breeding for diversification of primary gene pool and genetic enhancement of grain legumes. *Frontiers in plant science* **4**: 309.

Shewry PR. 2007. Improving the protein content and composition of cereal grain. *Journal of Cereal Science* **46**: 239–250.

Shewry PR. 2009. Wheat. *Journal of experimental botany* **60**: 1537–1553.

Shewry PR, Hey SJ. 2015. The contribution of wheat to human diet and health. *Food and energy security* **4**: 178–202.

Simonetti MC, Bellomo MP, Laghetti G, Perrino P, Simeone R, Blanco A. 1999. Quantitative trait loci influencing free-threshing habit in tetraploid wheats. *Genetic Resources and Crop Evolution* **46**: 267–271.

Simons KJ, Fellers JP, Trick HN, Zhang Z, Tai Y-S, Gill BS, Faris JD. 2006. Molecular characterization of the major wheat domestication gene Q. *Genetics* **172**: 547–555.

Smýkal P, Nelson M, Berger J, Wettberg E von. **2018.** The Impact of Genetic Changes during Crop Domestication. *Agronomy* **8**: 119.

Sood S, Kuraparthy V, Bai G, Gill BS. 2009. The major threshability genes soft glume (sog) and tenacious glume (Tg), of diploid and polyploid wheat, trace their origin to independent mutations at non-orthologous loci. *Theoretical and Applied Genetics* **119**: 341–351.

Stetter MG, Gates DJ, Mei W, Ross-Ibarra J. 2017. How to make a domesticate. *Current biology : CB* **27**: R896-R900.

Sukumaran S, Rebetzke G, Mackay I, Bentley AR, Reynolds MP. 2022. Pre-breeding Strategies. In: Reynolds MP, Braun H-J, eds. *Wheat Improvement*. Cham: Springer International Publishing, 451–469.

Tadesse W, Sachez-Garcia M, Assefa SG, Amri A, Bishaw Z, Ogbonnaya F, Baum M. 2019. Genetic Gains in Wheat Breeding and Its Role in Feeding the World. *Crop Breeding, Genetics and Genomics*. doi: 10.20900/cbgg20190005.

Talini RF, Brandolini A, Miculan M, Brunazzi A, Vaccino P, Pè ME, Dell'Acqua M. 2020. Genome-wide association study of agronomic and quality traits in a world collection of the wild wheat relative Triticum urartu. *The Plant Journal* **102**: 555–568.

Tanksley SD, McCouch SR. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. *Science (New York, N.Y.)* **277**: 1063–1066.

The Tomato Genome Consortium. 2012. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* **485**: 635–641.

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J. 2006. A NAC Gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science (New York, N.Y.)* **314**: 1298–1301.

Vestergaard NF, Jørgensen LN. 2024. Variety mixtures of winter wheat: a general status and national case study. *Journal of Plant Diseases and Protection*. doi: 10.1007/s41348-023-00856-z.

Waters BM, Uauy C, Dubcovsky J, Grusak MA. 2009. Wheat (Triticum aestivum) NAM proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. *Journal of experimental botany* **60**: 4263–4274.

Weiner J, Griepentrog H-W, KRISTENSEN L. 2001. Suppression of weeds by spring wheat Triticumaestivum increases with crop density and spatial uniformity. *Journal of Applied Ecology* **38**: 784–790.

Weiss E, Kislev ME, Hartmann A. 2006. Anthropology. Autonomous cultivation before domestication. *Science* **312**: 1608–1610.

Wieser H. 2007. Chemistry of gluten proteins. *Food microbiology* **24**: 115–119.

Willcox G. 2005. The distribution, natural habitats and availability of wild cereals in relation to their domestication in the Near East: multiple events, multiple centres. *Vegetation History and Archaeobotany* **14**: 534–541.

Wuest SE, Peter R, Niklaus PA. 2021. Ecological and evolutionary approaches to improving crop variety mixtures. *Nature ecology & evolution* **5**: 1068–1077.

Würschum T, Langer SM, Longin CFH, Tucker MR, Leiser WL. 2017. A modern Green Revolution gene for reduced height in wheat. *The Plant Journal* **92**: 892–903.

Yu H, Li J. 2022. Breeding future crops to feed the world through de novo domestication. *Nature Communications* **13**: 1171.

Yu H, Lin T, Meng X, Du H, Zhang J, Liu G, Chen M, Jing Y, Kou L, Li X *et al.* 2021. A route to de novo domestication of wild allotetraploid rice. *Cell*. doi: 10.1016/j.cell.2021.01.013.

Zahra N, Hafeez MB, Wahid A, Al Masruri MH, Ullah A, Siddique KHM, Farooq M. 2023. Impact of climate change on wheat grain composition and quality. *Journal of the Science of Food and Agriculture* **103**: 2745–2751.

Zeder MA. 2006. Central questions in the domestication of plants and animals. *Evolutionary Anthropology: Issues, News, and Reviews* **15**: 105–117.

Zeibig F, Kilian B, Frei M. 2022. The grain quality of wheat wild relatives in the evolutionary context. *Theoretical and Applied Genetics*. doi: 10.1007/s00122-021-04013-8.

Zeibig F, Kilian B, Özkan H, Pantha S, Frei M. 2024a. Phenotyping and identification of target traits for de novo domestication of wheat wild relatives. *Food and energy security*. doi: 10.1002/fes3.497.

Zeibig F, Kilian B, Özkan H, Pantha S, Frei M. 2024b. Grain quality traits within the wheat (Triticum spp.) genepool: prospects for improved nutrition through de novo domestication. *Journal of the Science of Food and Agriculture*. doi: 10.1002/jsfa.13328.

Zeng X, Mishina K, Jia J, Distelfeld A, Maughan PJ, Kikuchi S, Sassa H, Komatsuda T. 2020. The Brittle Rachis Trait in Species Belonging to the Triticeae and Its Controlling Genes Btr1 and Btr2. *Frontiers in plant science* **11**: 1000.

Zhang Z, Belcram H, Gornicki P, Charles M, Just J, Huneau C, Magdelenat G, Couloux A, Samain S, Gill BS *et al.* 2011. Duplication and partitioning in evolution and function of homoeologous Q loci governing domestication characters in polyploid wheat. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 18737–18742.

Zhou X, Zhao Y, Ni P, Ni Z, Sun Q, Zong Y. 2023. CRISPR-mediated acceleration of wheat improvement: advances and perspectives. *Journal of genetics and genomics = Yi chuan xue bao* **50**: 815–834.

Zohary D. 2004. Unconscious Selection and the Evolution of Domesticated Plants. *Economic Botany* **58**: 5–10.

Zsögön A, Čermák T, Naves ER, Notini MM, Edel KH, Weinl S, Freschi L, Voytas DF, Kudla J, Peres LEP. 2018. De novo domestication of wild tomato using genome editing. *Nature biotechnology*. doi: 10.1038/nbt.4272.

Zsögön A, Peres LEP, Xiao Y, Yan J, Fernie AR. 2022. Enhancing crop diversity for food security in the face of climate uncertainty. *The Plant Journal* **109**: 402–414.

Appendix

Appendix A

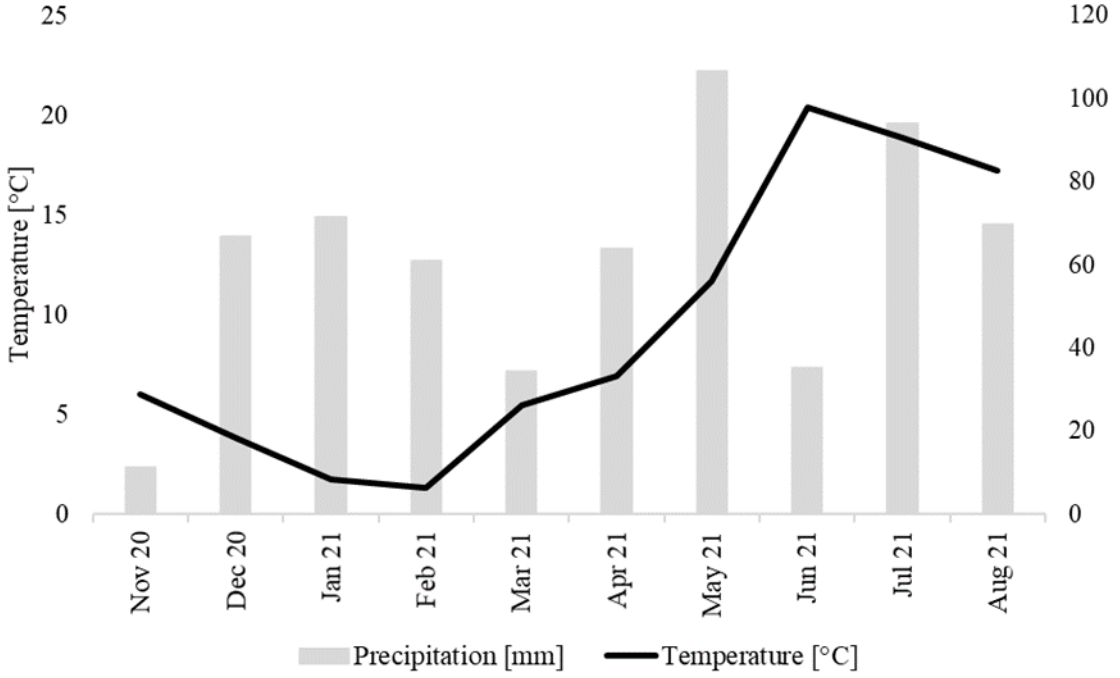

Figure SI 1: Monthly mean temperature and monthly precipitation sum from November 2020 until August 2021 at the research station at Giessen, Germany.

Black line represents monthly mean temperature; bars show total monthly precipitation in mm.

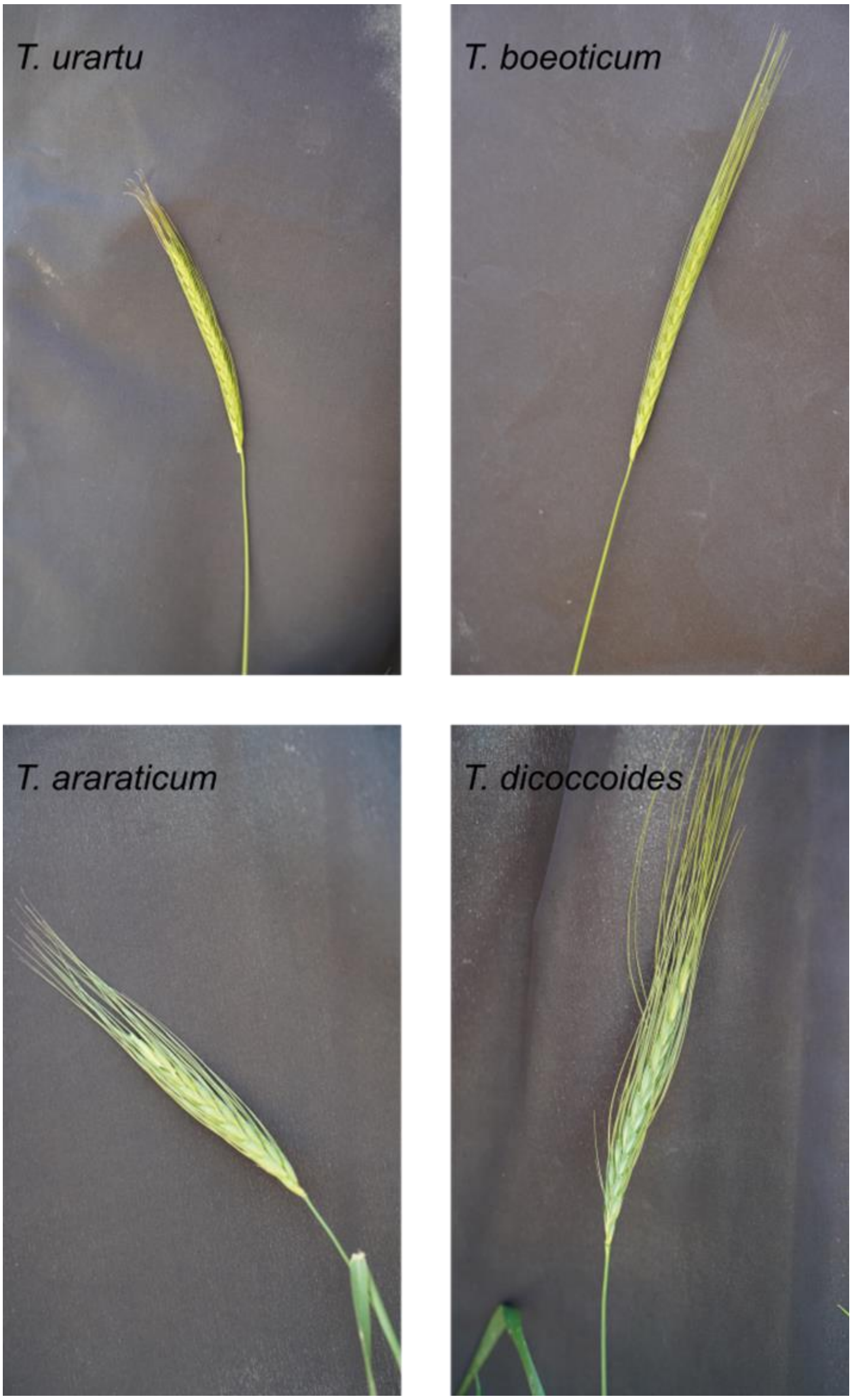

Figure SI 2: Spikes of the four known wild wheat taxa *T. urartu, T. boeoticum*, *T. araraticum*, and *T. dicoccoides* after completion of anthesis (Z69).

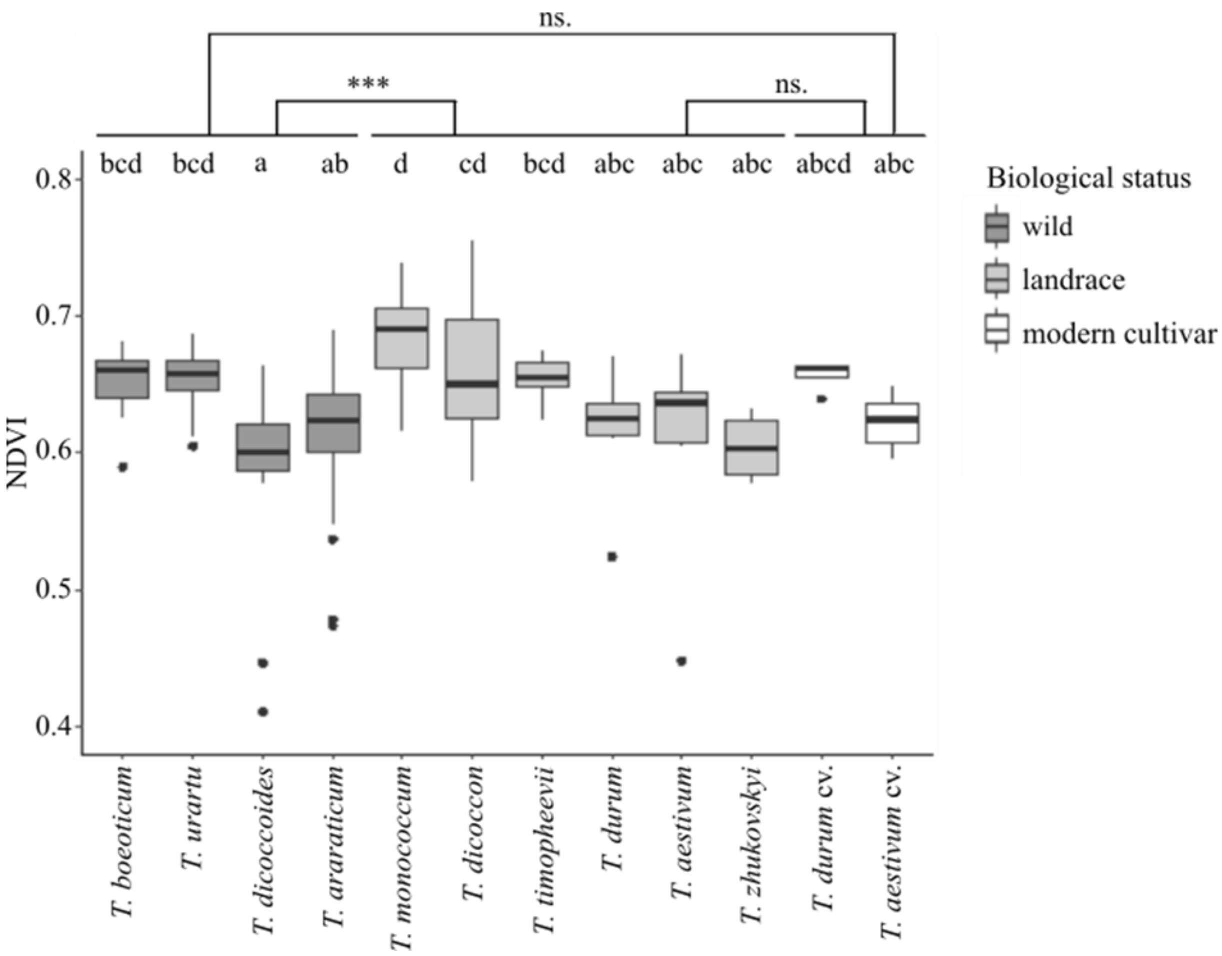

Figure SI 3: NDVI for wild taxa, landraces, and modern cultivars.

Shading indicates biological status group. Asterisks indicate significance between biological status groups (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$), ns. Indicates no significant difference, and different letters indicate significant differences among taxa ($p < 0.05$).

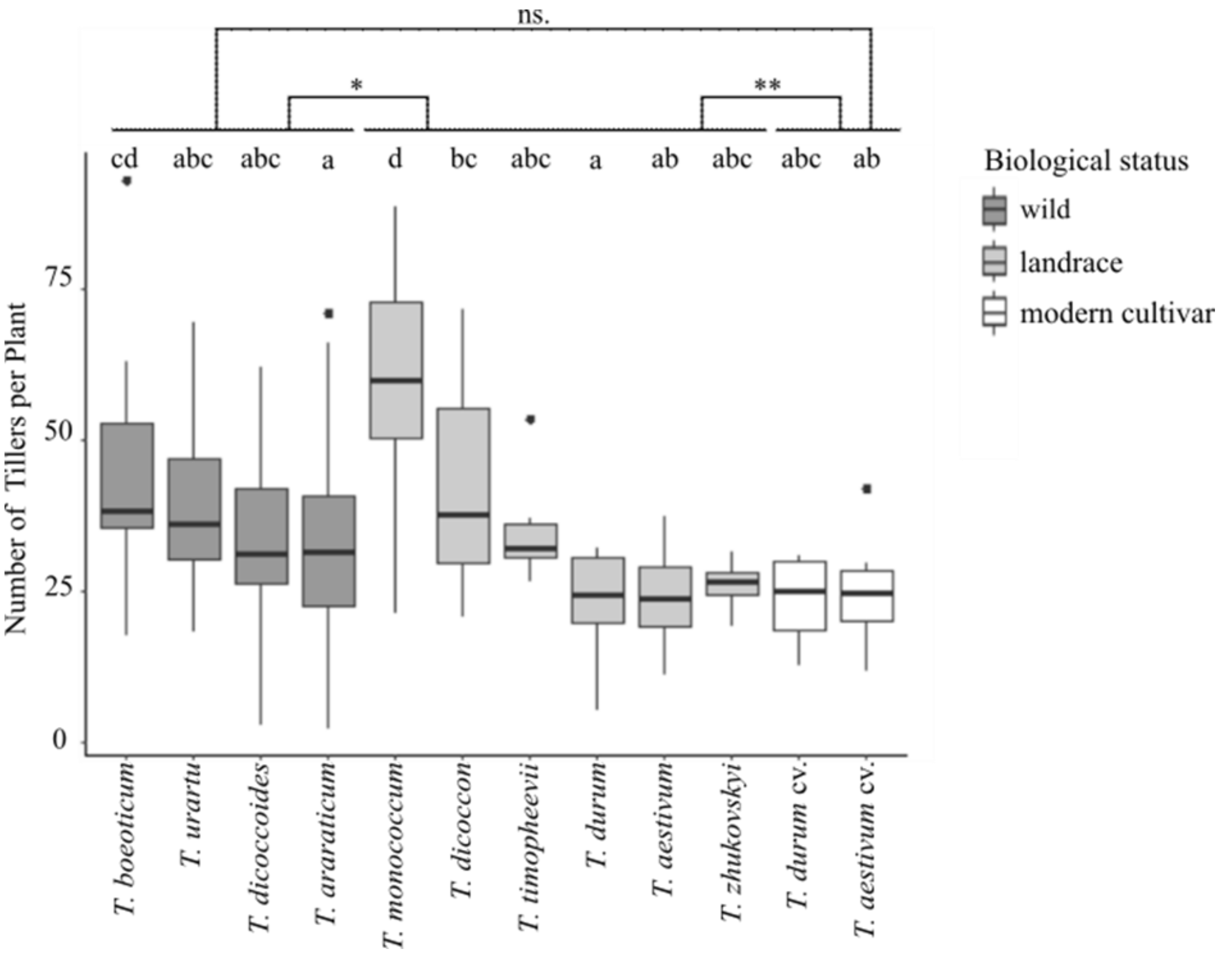

Figure SI 4: Number of tillers per plant for wild taxa, landraces, and modern cultivars. Shading indicates biological status group. Asterisks indicate significance between biological status groups (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$), ns. Indicates no significant difference, and different letters indicate significant differences among taxa ($p < 0.05$).

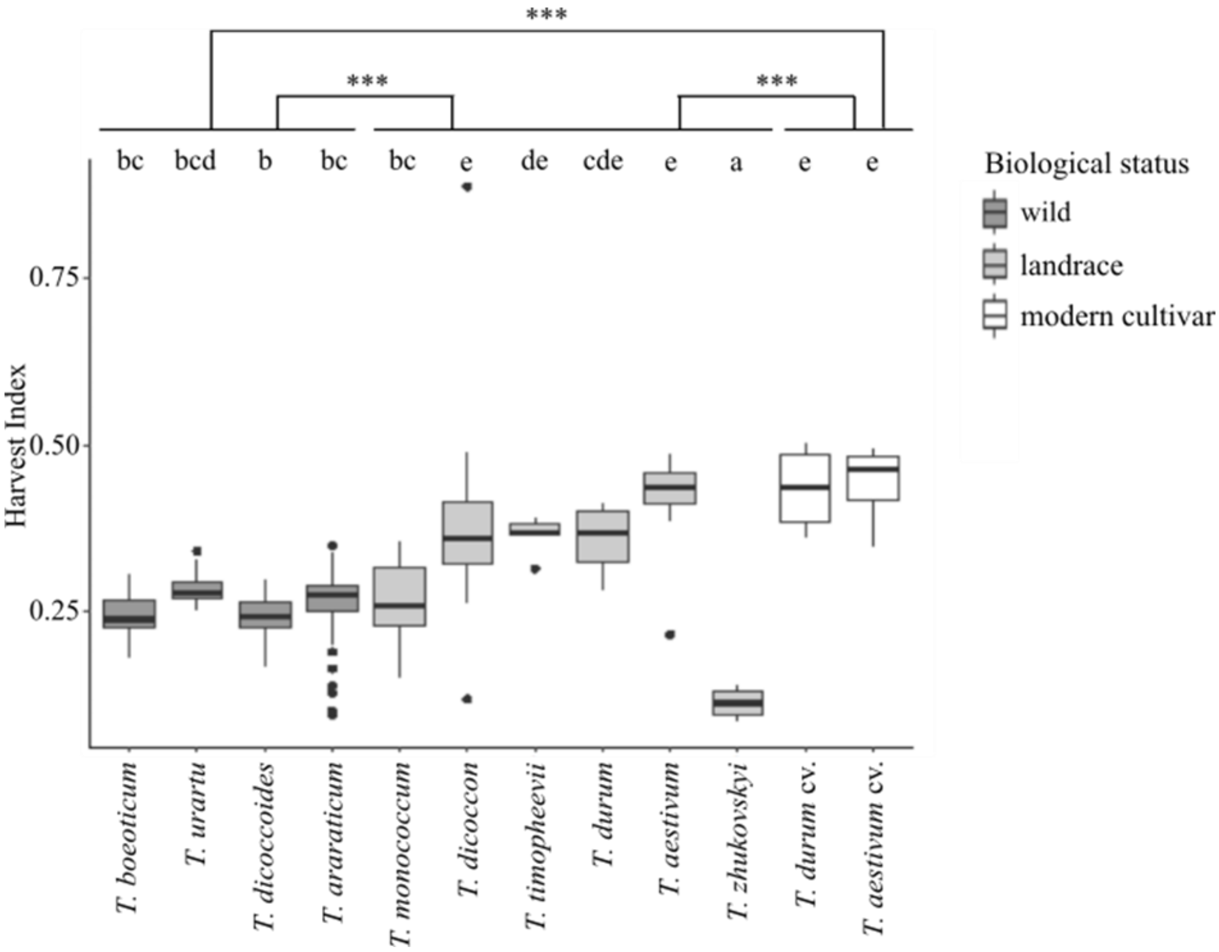

Figure SI 5: Harvest Index (HI) of wild taxa, landraces, and modern cultivars.

Shading indicates biological status group. Asterisks indicate significant difference between different biological status groups (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$), ns. indicates no significant differences, and different letters indicate significant differences among taxa ($p < 0.05$).

Table SI 1: Mean values of measured phenotypic parameters for each genotype

Geno-type Number	**Genus**	**Taxon**	**Genome**	**Origin**	**No. of tillers**	**NDVI**	**Heading**	**Anthesis**	**Stem height (cm)**	**TKW (g)**	**Hulless grain weight (g)**	**Hulled Grain Weight (g)**	**Harvest Index**
2	*Triticum*	*T. dicoccoides*	BA	TUR	59.0	0.60	1409	1529	98.8	19.8	19.32	45.91	0.22
3	*Triticum*	*T. aestivum*	BAD	OMN	34.3	0.64	1333	1446	101.5	39.4	40.32	62.50	0.44
5	*Triticum*	*T. urartu*	Au	LBN	32.0	0.67	1478	1563	89.3	10.7	12.59	17.70	0.33
7	*Triticum*	*T. timopheevii*	GA	SU	43.3	0.64	1573	1721	127.8	42.3	55.50	76.38	0.34
8	*Triticum*	*T. araraticum*	GA	IRN	28.8	0.61	1342	1482	89.2	26.1	13.81	26.22	0.29
10	*Triticum*	*T. aestivum*	BAD	AFG	16.8	0.61	1333	1472	89.9	26.8	28.84	29.70	0.33
11	*Triticum*	*T. araraticum*	GA	IRQ	20.0	0.61	1342	1487	94.4	20.6	9.68	22.77	0.24
12	*Triticum*	*T. araraticum*	GA	TUR	38.5	0.65	1371	1508	96.1	27.4	26.05	51.13	0.28
14	*Triticum*	*T. araraticum*	GA	IRQ	53.8	0.60	1258	1428	NA	22.0	15.98	47.53	0.23
15	*Triticum*	*T. monococcum*	Am	TUR	69.5	0.70	1492	1644	111.4	14.4	29.21	56.93	0.25
16	*Triticum*	*T. dicoccon*	BA	RUS	29.8	0.70	1478	1583	123.8	NA	32.45	49.44	0.35
17	*Triticum*	*T. urartu*	Au	TUR	48.3	0.67	1331	1439	111.0	12.1	16.16	29.17	0.26
18	*Triticum*	*T. boeoticum*	Ab	LBN	58.0	0.65	1400	1540	119.0	NA	NA	53.35	NA
22	*Triticum*	*T. dicoccoides*	BA	ISR	31.8	0.58	1318	1438	118.7	33.7	15.42	49.80	0.21
23	*Triticum*	*T. araraticum*	GA	IRN	41.8	0.58	1351	1508	91.5	22.1	14.75	30.01	0.26
24	*Triticum*	*T. zhukovskyi*	GAA	GEO	25.5	0.58	1619	1743	109.6	27.1	7.33	18.35	0.11
25	*Triticum*	*T. monococcum*	Am	TUR	67.3	0.70	1467	1597	113.3	18.2	23.52	50.46	0.21
27	*Triticum*	*T. boeoticum*	Ab	TUR	38.3	0.67	1325	1487	134.0	14.5	17.67	40.02	0.21
30	*Triticum*	*T. araraticum*	GA	TUR	37.8	0.65	1419	1540	96.9	30.4	23.73	49.27	0.29
31	*Triticum*	*T. araraticum*	GA	IRQ	35.5	0.61	1366	1492	94.9	22.2	17.92	37.39	0.27
32	*Triticum*	*T. araraticum*	GA	TUR	34.0	0.58	1286	1438	90.3	NA	NA	35.36	NA
33	*Triticum*	*T. boeoticum*	Ab	TUR	34.0	0.67	1414	1553	111.8	13.7	20.14	39.24	0.25
36	*Triticum*	*T. araraticum*	GA	AZJ	35.8	0.62	1513	1626	102.8	23.3	14.02	31.31	0.26
38	*Triticum*	*T. araraticum*	GA	SYR	39.3	0.65	1320	1471	88.3	29.4	20.51	58.65	0.26
39	*Triticum*	*T. araraticum*	GA	TUR	51.0	0.60	1353	1503	93.5	23.9	19.55	45.17	0.27
40	*Triticum*	*T. boeoticum*	Ab	TUR	44.0	0.66	1312	1477	106.9	14.8	20.18	38.86	0.22

Geno-type Number	**Genus**	**Taxon**	**Genome**	**Origin**	**No. of tillers**	**NDVI**	**Heading**	**Anthesis**	**Stem height (cm)**	**TKW (g)**	**Hulless grain weight (g)**	**Hulled Grain Weight (g)**	**Harvest Index**
41	*Triticum*	*T. durum cv.*	BAD	NA	30.3	0.66	1400	1539	63.6	33.7	52.23	68.97	0.45
42	*Triticum*	*T. araraticum*	GA	ARM	13.5	0.64	1437	1509	106.5	31.0	12.19	21.77	0.25
44	*Triticum*	*T. araraticum*	GA	TUR	32.3	0.66	1466	1567	105.1	32.4	29.69	52.56	0.29
45	*Triticum*	*T. urartu*	Au	SYR	36.3	0.63	1254	1381	108.5	10.1	14.91	28.85	0.29
47	*Triticum*	*T. araraticum*	GA	TUR	39.3	0.62	1362	1498	98.6	29.0	30.14	55.21	0.26
52	*Triticum*	*T. araraticum*	GA	IRQ	39.8	0.64	1275	1433	104.5	25.0	24.65	47.64	0.28
53	*Triticum*	*T. araraticum*	GA	TUR	33.5	0.67	1380	1503	85.5	27.9	17.60	35.63	0.31
55	*Triticum*	*T. aestivum*	BAD	ESP	18.5	0.65	1372	1478	99.3	47.2	27.94	33.40	0.44
56	*Triticum*	*T. araraticum*	GA	TUR	41.5	0.61	1366	1498	97.3	NA	NA	51.76	NA
57	*Triticum*	*T. araraticum*	GA	NA	30.5	0.61	1365	1492	85.8	27.8	11.34	26.85	0.29
59	*Triticum*	*T. araraticum*	GA	IRQ	30.5	0.59	1329	1457	91.8	25.1	14.85	34.66	0.27
60	*Triticum*	*T. araraticum*	GA	TUR	28.8	0.59	1333	1471	82.9	24.5	16.88	29.84	0.28
61	*Triticum*	*T. araraticum*	GA	IRQ	43.0	0.60	1321	1466	86.5	24.7	19.30	34.71	0.28
62	*Triticum*	*T. boeoticum*	Ab	TUR	26.5	0.62	1518	1626	100.0	13.7	13.38	18.00	0.25
63	*Triticum*	*T. araraticum*	GA	IRQ	24.5	0.59	1330	1463	90.9	NA	NA	14.29	NA
64	*Triticum*	*T. araraticum*	GA	TUR	35.3	0.65	1395	1535	93.4	30.0	18.69	37.43	0.29
65	*Triticum*	*T. dicoccon*	BA	TUR	58.0	0.70	1442	1549	99.3	27.5	32.08	44.12	0.38
66	*Triticum*	*T. boeoticum*	Ab	HUN	41.3	0.64	1508	1656	106.4	12.5	18.54	29.51	0.30
67	*Triticum*	*T. araraticum*	GA	IRQ	18.8	0.60	1351	1482	94.5	30.0	9.39	25.91	0.22
69	*Triticum*	*T. araraticum*	GA	SYR	29.5	0.64	1375	1498	79.3	23.3	10.28	26.86	0.20
70	*Triticum*	*T. araraticum*	GA	TUR	50.0	0.68	1462	1568	123.6	28.1	28.81	62.05	0.26
71	*Triticum*	*T. araraticum*	GA	TUR	20.3	0.52	1419	1498	71.6	11.3	4.40	12.61	0.13
73	*Triticum*	*T. dicoccon*	BA	MAR	24.0	0.59	1342	1456	76.8	NA	NA	37.66	NA
77	*Triticum*	*T. dicoccon*	BA	ISR	31.5	0.63	1609	1710	108.8	44.5	70.1	90.9	0.3
79	*Triticum*	*T. urartu*	Au	IRN	24.0	0.66	1299	1410	103.6	11.3	9.2	17.6	0.3
81	*Triticum*	*T. urartu*	Au	IRN	33.0	0.66	1325	1447	112.3	NA	NA	23.1	NA
82	*Triticum*	*T. durum*	BA	MAR	23.5	0.67	1456	1568	96.5	NA	NA	61.6	NA

Geno-type Number	**Genus**	**Taxon**	**Genome**	**Origin**	**No. of tillers**	**NDVI**	**Heading**	**Anthesis**	**Stem height (cm)**	**TKW (g)**	**Hulless grain weight (g)**	**Hulled Grain Weight (g)**	**Harvest Index**
83	*Triticum*	*T. araraticum*	GA	IRQ	29.0	0.64	1287	1429	84.6	22.7	13.2	21.4	0.3
84	*Triticum*	*T. dicoccon*	BA	ROM	46.5	0.62	1675	1744	122.3	31.0	62.1	99.2	0.3
85	*Triticum*	*T. dicoccoides*	BA	ISR	21.5	0.60	1382	1528	86.2	NA	NA	41.7	NA
90	*Triticum*	*T. dicoccoides*	BA	TUR	41.0	0.60	1395	1492	97.0	29.2	21.5	47.9	0.2
91	*Triticum*	*T. araraticum*	GA	NA	30.0	0.64	1349	1483	91.6	30.8	14.3	33.1	0.2
92	*Triticum*	*T. durum*	BA	FRA	20.8	0.62	1424	1552	53.9	35.7	24.2	34.8	0.3
93	*Triticum*	*T. timopheevii*	GA	SU	30.8	0.66	1577	1721	118.1	44.8	36.3	50.1	0.4
95	*Triticum*	*T. timopheevii*	GA	TUR	32.0	0.66	1512	1657	119.9	44.9	43.3	61.6	0.4
99	*Triticum*	*T. araraticum*	GA	IRN	34.8	0.63	1326	1458	88.0	22.5	14.4	24.2	0.3
101	*Triticum*	*T. dicoccoides*	BA	SYR	36.8	0.61	1334	1458	96.6	27.9	27.5	55.5	0.3
102	*Triticum*	*T. araraticum*	GA	IS	22.8	0.59	1269	1410	98.0	33.9	21.5	49.1	0.3
103	*Triticum*	*T. araraticum*	GA	TUR	35.3	0.59	1390	1519	83.6	29.2	24.7	46.6	0.3
104	*Triticum*	*T. araraticum*	GA	IRQ	25.5	0.62	1380	1514	83.0	27.7	12.7	22.3	0.3
105	*Triticum*	*T. monococcum*	Am	GRC	42.5	0.65	1533	1657	96.5	18.6	22.1	32.6	0.3
107	*Triticum*	*T. araraticum*	GA	IRQ	35.5	0.63	1305	1443	93.3	27.0	15.8	27.9	0.3
108	*Triticum*	*T. zhukovskyi*	GAA	GEO	26.5	0.63	1597	1739	105.0	40.4	7.6	20.6	0.1
112	*Triticum*	*T. dicoccon*	BA	ESP	34.5	0.64	1530	1607	120.1	54.8	58.4	75.1	0.4
114	*Triticum*	*T. araraticum*	GA	IRN	39.5	0.59	1351	1487	91.5	NA	NA	27.1	NA
117	*Triticum*	*T. araraticum*	GA	TUR	32.8	0.64	1410	1957	83.6	24.3	17.2	31.8	0.3
118	*Triticum*	*T. dicoccoides*	BA	LBN	29.8	0.62	1309	1433	93.8	29.9	23.7	45.7	0.3
120	*Triticum*	*T. araraticum*	GA	TUR	35.5	0.62	1381	1498	99.5	NA	NA	37.5	NA
121	*Triticum*	*T. araraticum*	GA	IRQ	39.5	0.62	1296	1442	97.8	23.0	19.3	47.9	0.3
122	*Triticum*	*T. dicoccon*	BA	IRN	54.0	0.66	1442	1549	116.1	38.4	52.8	56.5	0.4
123	*Triticum*	*T. araraticum*	GA	IRQ	13.8	0.60	1320	1428	58.4	NA	NA	8.5	NA
125	*Triticum*	*T. araraticum*	GA	IRQ	32.3	0.58	1285	1419	96.3	NA	NA	37.8	NA
128	*Triticum*	*T. araraticum*	GA	IRQ	32.3	0.61	1314	1442	76.6	24.3	16.1	30.9	0.3
129	*Triticum*	*T. araraticum*	GA	IRQ	13.0	0.61	1437	1549	68.0	NA	NA	12.7	NA

Geno-type Number	**Genus**	**Taxon**	**Genome**	**Origin**	**No. of tillers**	**NDVI**	**Heading**	**Anthesis**	**Stem height (cm)**	**TKW (g)**	**Hulless grain weight (g)**	**Hulled Grain Weight (g)**	**Harvest Index**
131	*Triticum*	*T. araraticum*	GA	IRQ	27.5	0.59	1294	1438	94.5	29.6	17.2	26.5	0.3
134	*Triticum*	*T. aestivum cv.*	BAD	NA	17.0	0.63	1419	1582	62.4	38.8	45.4	52.2	0.5
139	*Triticum*	*T. araraticum*	GA	IRQ	27.8	0.60	1325	1463	81.3	26.7	21.3	35.7	0.3
140	*Triticum*	*T. araraticum*	GA	IRN	45.0	0.59	1304	1462	97.4	21.3	19.2	42.1	0.3
141	*Triticum*	*T. araraticum*	GA	IRN	30.5	0.62	1414	1525	105.5	30.9	24.0	41.6	0.3
142	*Triticum*	*T. araraticum*	GA	IRQ	25.0	0.62	1380	1512	96.0	29.2	15.4	36.2	0.3
143	*Triticum*	*T. araraticum*	GA	IRQ	30.5	0.59	1254	1405	107.8	25.8	18.5	36.8	0.3
144	*Triticum*	*T. araraticum*	GA	TUR	14.0	0.64	1342	1482	77.8	29.7	8.5	18.1	0.2
145	*Triticum*	*T. dicoccon*	BA	IND	37.5	0.61	1318	1444	98.3	30.8	33.9	50.7	0.4
146	*Triticum*	*T. aestivum cv.*	BAD	NA	26.5	0.64	1424	1574	60.1	38.8	42.0	50.5	0.4
151	*Triticum*	*T. araraticum*	GA	IRQ	26.8	0.64	1321	1477	105.9	23.8	16.8	33.1	0.3
153	*Triticum*	*T. araraticum*	GA	IRQ	37.0	0.64	1215	1400	96.8	29.5	18.2	35.7	0.3
154	*Triticum*	*T. araraticum*	GA	TUR	26.3	0.68	1268	1404	101.8	10.5	12.5	19.2	0.3
155	*Triticum*	*T. araraticum*	GA	TUR	38.0	0.61	1361	1498	99.8	29.6	22.8	39.5	0.3
156	*Triticum*	*T. araraticum*	GA	TUR	23.8	0.60	1512	1621	100.8	29.6	19.6	32.4	0.3
158	*Triticum*	*T. araraticum*	GA	IRQ	30.0	0.66	1279	1438	85.8	25.5	12.0	28.4	0.3
160	*Triticum*	*T. aestivum*	BAD	IRN	27.0	0.55	1458	1558	92.1	39.4	62.8	55.1	0.4
161	*Triticum*	*T. araraticum*	GA	IRQ	29.0	0.58	1321	1452	95.6	27.5	23.5	39.6	0.3
165	*Triticum*	*T. dicoccon*	BA	ETH	46.5	0.65	1275	1419	92.6	42.8	27.9	53.9	0.2
166	*Triticum*	*T. urartu*	Au	TUR	58.3	0.62	1453	1583	116.3	13.5	17.0	36.5	0.3
167	*Triticum*	*T. durum cv.*	BA	NA	16.8	0.65	1424	1528	73.9	30.2	37.1	40.5	0.4
169	*Triticum*	*T. aestivum cv.*	BAD	NA	21.0	0.62	1429	1539	57.2	44.5	43.9	50.3	0.4
172	*Triticum*	*T. araraticum*	GA	IRQ	34.3	0.61	1371	1503	100.0	NA	NA	43.1	NA
174	*Triticum*	*T. monococcum*	Am	MAR	23.5	0.66	1472	1621	77.6	24.7	17.0	18.5	0.3
177	*Triticum*	*T. araraticum*	GA	TUR	28.5	0.64	1386	1856	92.0	30.3	20.9	35.9	0.3
180	*Triticum*	*T. araraticum*	GA	IRQ	40.8	0.58	1285	1419	92.2	28.4	15.2	35.4	0.3
181	*Triticum*	*T. aestivum cv.*	BAD	NA	34.0	0.60	1395	1572	65.3	42.4	86.7	91.9	0.5

Geno-type Number	**Genus**	**Taxon**	**Genome**	**Origin**	**No. of tillers**	**NDVI**	**Heading**	**Anthesis**	**Stem height (cm)**	**TKW (g)**	**Hulless grain weight (g)**	**Hulled Grain Weight (g)**	**Harvest Index**
182	*Triticum*	*T. monococcum*	Am	TUR	70.8	0.66	1559	1675	109.3	22.7	23.0	42.0	0.2
185	*Triticum*	*T. araraticum*	GA	TUR	56.5	0.63	1390	1508	106.5	29.9	23.3	51.0	0.3
186	*Triticum*	*T. araraticum*	GA	IRN	36.3	0.59	1308	1447	89.0	27.0	18.0	34.6	0.3
188	*Triticum*	*T. durum*	BA	IND	18.8	0.57	1361	1487	72.4	NA	NA	32.7	NA
189	*Triticum*	*T. araraticum*	GA	IRN	38.5	0.62	1326	1478	90.4	24.9	18.4	30.9	0.2
191	*Triticum*	*T. araraticum*	GA	NA	37.5	0.61	1390	1519	99.5	NA	NA	53.5	NA
192	*Triticum*	*T. monococcum*	Am	ESP	72.3	0.74	1462	1587	117.1	19.4	32.1	63.2	0.2
193	*Triticum*	*T. araraticum*	GA	SYR	23.5	0.67	1272	1456	81.0	19.5	12.1	27.3	0.2
194	*Triticum*	*T. araraticum*	GA	IRQ	32.0	0.62	1322	1462	78.4	23.2	16.3	26.5	0.3
195	*Triticum*	*T. monococcum*	Am	ITA	57.5	0.68	1457	1593	128.3	24.2	46.0	59.3	0.3
197	*Triticum*	*T. araraticum*	GA	IRQ	15.0	0.56	1362	1503	77.8	NA	NA	21.9	NA
198	*Triticum*	*T. dicoccon*	BA	SYR	29.3	0.68	1498	1633	119.1	44.3	40.5	69.0	0.3
199	*Triticum*	*T. araraticum*	GA	IRQ	39.3	0.64	1329	1452	96.8	28.4	18.5	44.8	0.3
200	*Triticum*	*T. araraticum*	GA	ARM	34.3	0.65	1535	1631	90.0	15.6	14.3	33.4	0.2
201	*Triticum*	*T. boeoticum*	Ab	TUR	73.5	0.65	1351	1508	98.4	12.9	25.7	53.4	0.3
202	*Triticum*	*T. araraticum*	GA	TUR	30.3	0.65	1424	1549	83.9	28.7	19.3	32.3	0.3
203	*Triticum*	*T. araraticum*	GA	IRQ	25.8	0.60	1330	1473	90.5	25.3	18.8	29.4	0.3
205	*Triticum*	*T. araraticum*	GA	IRQ	17.8	0.62	1385	1508	70.1	28.2	15.8	21.3	0.3
206	*Triticum*	*T. dicoccoides*	BA	IRN	14.3	0.43	1383	1470	48.0	NA	NA	8.6	NA
208	*Triticum*	*T. dicoccon*	BA	TUR	51.5	0.70	1492	1587	100.8	31.3	46.9	49.9	0.4
210	*Triticum*	*T. durum*	BA	ITA	31.0	0.62	1423	1524	124.8	57.6	86.1	122.8	0.4
215	*Triticum*	*T. araraticum*	GA	IRQ	26.3	0.63	1385	1529	94.4	30.9	26.5	38.1	0.3
217	*Triticum*	*T. araraticum*	GA	TUR	24.5	0.64	1414	1549	91.8	21.2	11.7	26.3	0.2
218	*Triticum*	*T. dicoccon*	BA	SVK	59.8	0.71	1452	1583	115.9	28.5	64.1	61.0	0.4
219	*Triticum*	*T. araraticum*	GA	TUR	19.0	0.66	1482	1598	100.5	31.2	14.1	30.1	0.3
221	*Triticum*	*T. araraticum*	GA	IRQ	21.5	0.59	1376	1507	66.9	26.2	13.1	21.0	0.3
222	*Triticum*	*T. araraticum*	GA	IRQ	23.0	0.60	1386	1512	NA	28.8	24.1	31.9	0.3

Geno-type Number	Genus	Taxon	Genome	Origin	No. of tillers	NDVI	Heading	Anthesis	Stem height (cm)	TKW (g)	Hulless grain weight (g)	Hulled Grain Weight (g)	Harvest Index
224	*Triticum*	*T. araraticum*	GA	TUR	46.5	0.63	1419	1540	111.0	NA	NA	64.9	NA
231	*Triticum*	*T. araraticum*	GA	TUR	35.5	0.64	1409	1540	87.6	25.8	20.8	38.0	0.3
232	*Triticum*	*T. araraticum*	GA	TUR	42.3	0.67	1477	1593	114.1	34.5	39.0	68.4	0.3
233	*Triticum*	*T. araraticum*	GA	TUR	27.3	0.64	1415	1535	92.0	28.4	17.5	33.7	0.3
236	*Triticum*	*T. araraticum*	GA	IRQ	26.3	0.62	1266	1410	76.3	NA	NA	16.5	NA
237	*Triticum*	*T. araraticum*	GA	ARM	18.0	0.64	1509	1620	66.8	NA	NA	12.6	NA
238	*Triticum*	*T. araraticum*	GA	IRQ	31.3	0.60	1331	1458	89.5	27.9	14.4	27.6	0.3
242	*Triticum*	*T. dicoccoides*	BA	ISR	22.5	0.62	1203	1400	107.5	32.6	18.3	41.4	0.2
243	*Triticum*	*T. dicoccon*	BA	OMN	37.5	0.68	1210	1356	87.0	33.9	43.6	40.1	0.7
244	*Triticum*	*T. araraticum*	GA	SYR	40.0	0.66	1343	1458	92.2	30.4	17.0	46.4	0.2
246	*Triticum*	*T. araraticum*	GA	IRQ	42.0	0.63	1285	1438	98.8	22.4	9.1	32.9	0.2
248	*Triticum*	*T. dicoccoides*	BA	IRQ	49.3	0.64	1260	1395	90.6	25.6	22.6	47.6	0.3

Appendix B

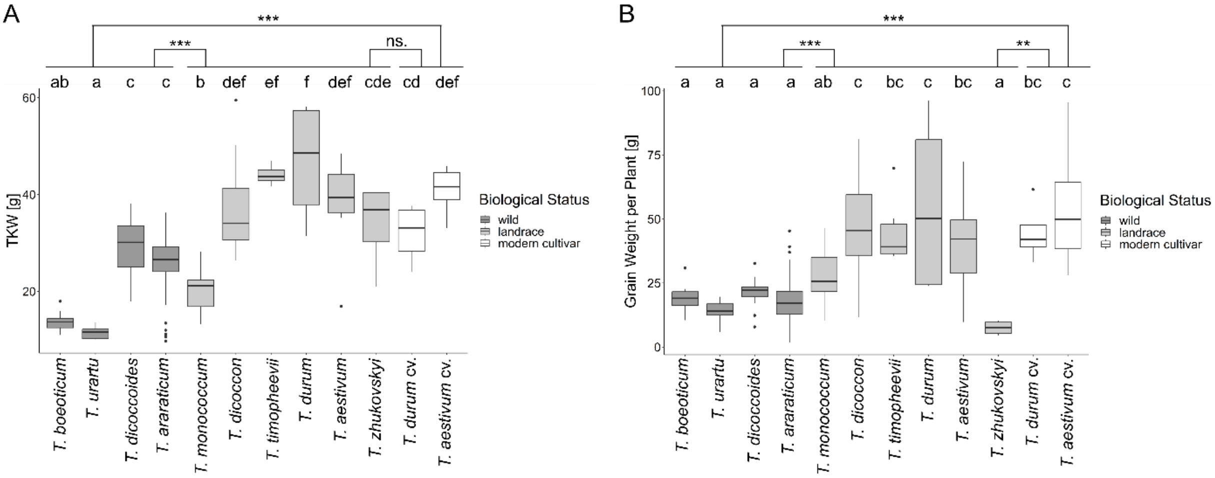

Figure SI 6: Yield data of wild taxa, landraces, and modern cultivars. (A) TKW (g) of wild taxa, landraces, and modern cultivars; (B) grain weight per plant (g) of wild taxa, landraces, and modern cultivars.

Asterisks indicate significant differences between groups according to their biological status (*p < 0.05: **p < 0.01; ***p < 0.001), ns. indicates no significant difference, and different letters indicate significant differences among taxa (p < 0.05). Data adapted from Zeibig *et al.* (2024a)

Table SI 2: Grain quality parameters for different wheat genotypes (mean values)

Geno-type no.	**Taxon**	**Genome**	**Original sample number**	**Other identifiers**	**Origin**	**Mean Fe (mg/kg)**	**Mean Zn (mg/kg)**	**Mean Phyt:Fe**	**Mean Phyt:Zn**	**Mean P (mg/g)**	**Mean phytate (mg/g)**	**Mean GPC (g/kg)**	**TPC (µmol GAE/g)**	**ORAC (µmol TE/g)**
2	*T. dicoccoides*	BA	PI 654339		TUR	86.37	47.91	9.51	20.07	6.51	9.69	224.5	4.61	21.18
3	*T. aestivum*	BAD	IG 45071		OMN	42.06	36.02	17.09	23.37	5.22	8.50	137.6	4.56	14.16
5	*T. urartu*	Au	G 3246, PI 428320	ID 388 EP047-1	LBN	62.20	44.82	13.05	21.40	6.66	9.59	192.0	4.75	21.47
7	*T. timopheevii*	GAt	KU-1820	TR 106 TP041-TP045	SU	56.85	46.81	13.02	18.68	5.75	8.75	170.2	3.75	9.58
8	*T. araraticum*	GAt	PI 427365	PI 427365; TR 133-1; TA 101	IRN	90.72	68.76	9.33	14.42	6.45	10.00	252.5	4.08	16.29
10	*T. aestivum*	BAD	PI 367199		AFG	47.83	46.01	17.58	20.78	6.76	9.65	167.0	4.86	18.23
11	*T. araraticum*	GAt	KU-8706	8706	IRQ	88.38	53.12	10.07	18.60	6.96	9.88	259.1	3.98	16.53
12	*T. araraticum*	GAt	KU-1960		TUR	63.13	43.60	12.24	20.70	6.30	9.11	210.2	3.75	16.40
14	*T. araraticum*	GAt	KU-8682	TR 61 TP071	IRQ	55.93	43.09	13.10	20.09	6.44	8.61	241.4	3.35	10.70
15	*T. monococcum*	Ab	PI 119435	ID 05 EP001-EP002	TUR	43.43	41.81	17.83	21.91	6.87	9.13	165.2	5.09	16.16
16	*T. dicoccon*	BA	PI 41025		RUS	50.41	53.98	14.65	15.96	6.26	8.70	195.4	4.17	17.33
17	*T. urartu*	Au	PI 428184	ID 1396 EP036-EP037	TUR	68.48	59.01	11.98	15.98	6.68	9.50	228.0	4.49	17.54
22	*T. dicoccoides*	BA	Gamla 1		ISR	57.44	69.87	13.38	12.93	6.34	8.99	206.5	3.97	15.00
23	*T. araraticum*	GAt	KU-8945	TR 101-1; KU-8945; 2761	IRN	66.17	57.17	12.04	16.32	7.16	9.41	246.8	3.98	15.23
24	*T. zhukovskyi*	GAtAb	TRI 7258		GEO	63.04	95.64	13.63	10.49	7.21	9.89	228.7	5.94	24.99

Geno-type no.	Taxon	Genome	Original sample number	Other identifiers	Origin	Mean Fe (mg/kg)	Mean Zn (mg/kg)	Mean Phyt:Fe	Mean Phyt:Zn	Mean P (mg/g)	Mean phytate (mg/g)	Mean GPC (g/kg)	TPC (µmol GAE/g)	ORAC (µmol TE/g)
25	*T. monococcum*	Ab	PI 119423	ID 492 EP049-EP057	TUR	37.26	36.07	19.33	23.37	5.88	8.51	161.5	3.95	17.83
27	*T. boeoticum*	Ab	PI 427583	ID 716 EP064-EP066	TUR	83.96	82.89	9.54	11.48	7.19	9.47	219.1	4.94	18.38
30	*T. araraticum*	GAt	KU-1982	2797	TUR	78.76	49.04	9.16	17.27	5.75	8.53	151.2	4.08	13.49
31	*T. araraticum*	GAt	KU-8774	KU-8774; TR80-4; 8774	IRQ	87.09	85.34	9.23	12.56	7.11	9.39	230.8	4.00	13.18
33	*T. boeoticum*	Ab	PI 427458	ID 597 EP062-EP063	TUR	86.48	60.25	9.67	16.29	8.33	9.88	233.2	4.75	20.24
36	*T. araraticum*	GAt	HTRI 11356/76	TR 122; 2765	AZJ	80.81	66.32	10.20	14.56	6.69	9.70	219.3	4.52	18.32
38	*T. araraticum*	GAt	IG 117891		SYR	105.36	55.01	7.73	17.02	6.58	9.45	221.5	4.44	12.28
39	*T. araraticum*	GAt	IG 116165	2661	TUR	66.16	55.52	12.50	17.52	7.29	9.76	225.0	4.51	15.18
40	*T. boeoticum*	Ab	PI 427629	ID 760 EP087-EP095	TUR	64.00	52.37	12.22	17.49	7.09	9.25	208.8	4.88	16.46
41	*T. durum* cv. Sambadur	BA	Hauptsaaten		NA	47.25	43.39	16.41	21.06	6.12	9.02	156.8	4.68	15.37
42	*T. araraticum*	GAt	KU-3627	1959-7-5-2; 1914	ARM	75.41	52.87	10.38	19.43	6.10	9.21	234.8	4.28	22.51
44	*T. araraticum*	GAt	KU-8939	TR 99 TP083-TP087	TUR	76.78	44.55	9.95	20.10	5.99	9.01	200.0	4.06	13.95
45	*T. urartu*	Au	IG 45285		SYR	78.00	43.50	9.85	20.68	6.22	9.08	202.6	5.06	17.45
47	*T. araraticum*	GAt	2006-6-20-2-2 D	2633	TUR	64.89	45.88	12.39	19.57	6.22	9.05	221.0	4.86	18.06
52	*T. araraticum*	GAt	PI 297030	2566	IRQ	61.00	63.54	13.93	14.70	7.01	9.43	240.0	4.08	13.93
53	*T. araraticum*	GAt	KU-1984B	2932	TUR	61.20	50.81	12.56	17.71	6.13	9.08	220.8	4.39	17.65

Geno-type no.	**Taxon**	**Genome**	**Original sample number**	**Other identifiers**	**Origin**	**Mean Fe (mg/kg)**	**Mean Zn (mg/kg)**	**Mean Phyt:Fe**	**Mean Phyt:Zn**	**Mean P (mg/g)**	**Mean phytate (mg/g)**	**Mean GPC (g/kg)**	**TPC (µmol GAE/g)**	**ORAC (µmol TE/g)**
55	*T. aestivum*	BAD	LPCH101	FAR 276; TR 183	ESP	30.64	71.71	25.74	12.86	6.37	9.31	196.8	4.20	11.94
57	*T. araraticum*	GAt	PI 221421	2554	NA	62.63	79.37	13.39	11.56	7.08	9.23	252.1	4.13	11.32
59	*T. araraticum*	GAt	PI 290518	2565	IRQ	78.11	63.37	10.35	14.98	6.63	9.55	245.2	4.33	17.50
60	*T. araraticum*	GAt	IG 116165		TUR	44.01	61.84	20.43	15.35	6.53	9.58	239.5	4.76	19.63
61	*T. araraticum*	GAt	KU-8682	TR 61 TP071; 2713	IRQ	49.86	65.19	16.73	14.97	5.81	9.85	247.6	4.39	12.78
62	*T. boeoticum*	Ab	PI 427963	ID 1089 EP003-EP004	TUR	78.78	65.45	10.48	14.58	6.72	9.63	223.9	4.72	18.76
64	*T. araraticum*	GAt	KU-1966	TR 44-2	TUR	62.38	54.75	12.76	16.94	6.10	9.36	226.8	4.45	15.47
65	*T. dicoccon*	BA	TRI 17023	DEU146:W 7700	TUR	45.35	44.01	16.53	19.85	6.12	8.78	171.0	4.62	16.05
66	*T. boeoticum*	Ab	PI 272520	ID 520 EP058-EP059	HUN	86.48	57.73	8.87	15.71	6.49	9.07	169.1	5.12	12.28
67	*T. araraticum*	GAt	KU-8946	2919	IRQ	71.74	52.87	10.88	19.00	6.16	9.03	234.9	4.62	13.58
69	*T. araraticum*	GAt	IG 119456	2684	SYR	101.16	68.00	8.25	14.31	6.78	9.64	264.1	4.83	17.03
70	*T. araraticum*	GAt	KU-1986	2798	TUR	55.24	51.82	14.50	18.37	6.28	9.43	217.4	4.60	12.89
71	*T. araraticum*	GAt	PI 428018	3307; TR15	TUR	70.12	66.87	11.00	13.47	6.55	9.10	245.2	5.92	32.22
77	*T. dicoccon*	BA	TRI 16880	DEU146:W 7561 B	ISR	52.03	45.28	14.05	18.94	6.13	8.63	155.4	4.54	18.29
79	*T. urartu*	Au	G 3221, PI 428317	ID 393 EP048-1	IRN	84.21	72.54	9.31	12.70	8.50	9.25	228.5	5.56	28.27
83	*T. araraticum*	GAt	KU-8691	2862	IRQ	86.16	65.35	10.24	15.86	7.28	10.43	252.3	4.88	17.46
84	*T. dicoccon*	BA	PI 362071		ROM	89.33	85.69	9.73	11.88	7.38	10.21	234.7	4.36	23.94

Geno-type no.	Taxon	Genome	Original sample number	Other identifiers	Origin	Mean Fe (mg/kg)	Mean Zn (mg/kg)	Mean Phyt:Fe	Mean Phyt:Zn	Mean P (mg/g)	Mean phytate (mg/g)	Mean GPC (g/kg)	TPC (µmol GAE/g)	ORAC (µmol TE/g)
90	*T. dicoccoides*	BA	KU-8935		TUR	51.41	66.53	15.92	14.19	6.19	9.53	166.3	4.48	23.62
91	*T. araraticum*	GAt	KU-8824A	1970-6-16-5-3-B-2	IRQ	115.79	83.26	7.51	12.18	9.44	10.18	250.8	4.34	20.35
92	*T. durum*	BA	DIC 169		FRA	53.93	61.23	15.33	15.83	6.73	9.78	168.2	3.79	13.07
93	*T. timopheevii*	GAt	KU-1821	TR 107 TP046-TP053	SU	64.84	55.93	11.33	14.60	6.59	8.22	173.3	3.75	14.00
95	*T. timopheevii*	GAt	PI 119442		TUR	51.20	46.73	11.57	14.92	5.96	7.00	167.2	3.87	16.63
99	*T. araraticum*	GAt	KU-8945	TR 101-1; 8945	IRN	74.05	68.29	10.03	12.71	7.72	8.78	250.9	4.38	17.18
101	*T. dicoccoides*	BA	KU-108-2		SYR	51.81	74.70	16.55	13.22	6.26	9.85	240.5	3.94	16.40
102	*T. dicoccoides*	BA	Arbel	3235	ISR	58.72	55.28	15.16	18.45	6.16	10.29	223.1	4.47	16.69
103	*T. araraticum*	GAt	KU-8910	TR 94 TP077-TP079	TUR	95.49	66.52	8.75	14.74	7.24	9.86	222.8	4.22	16.21
104	*T. araraticum*	GAt	KU-8788	8788	IRQ	76.60	67.00	10.95	14.66	8.90	9.90	223.7	4.04	16.86
105	*T. monococcum*	Ab	PI 277137	ID 1690	GRC	64.31	56.55	9.54	13.00	6.53	7.25	179.3	5.02	17.82
107	*T. araraticum*	GAt	KU-8912	2879	IRQ	87.17	51.28	9.25	19.33	7.38	9.53	251.6	4.19	12.11
108	*T. zhukovskyi*	GAtAb	A TRI 12094/80	FAR 78; TR 156	GEO	83.63	123.59	8.53	6.77	10.71	8.42	248.5	6.70	26.60
112	*T. dicoccon*	BA	PI 276002		ESP	55.82	52.12	14.69	18.11	6.46	9.42	179.5	3.88	11.66
117	*T. araraticum*	GAt	2006-6-20-11-6 D	2643	TUR	73.04	62.52	12.11	16.62	6.86	10.42	228.0	4.44	15.12
118	*T. dicoccoides*	BA	PI 352322	DIC 44 TP011-TP020	LBN	68.18	42.42	10.87	20.48	7.18	8.76	213.0	4.21	17.04

Geno-type no.	Taxon	Genome	Original sample number	Other identifiers	Origin	Mean Fe (mg/kg)	Mean Zn (mg/kg)	Mean Phyt:Fe	Mean Phyt:Zn	Mean P (mg/g)	Mean phytate (mg/g)	Mean GPC (g/kg)	TPC (µmol GAE/g)	ORAC (µmol TE/g)
121	*T. araraticum*	GAt	PI 538461	TR 17 TP061-TP063	IRQ	104.29	56.99	7.98	17.23	8.24	9.83	254.3	4.17	15.89
122	*T. dicoccon*	BA	TRI 5860		IRN	48.24	42.52	16.49	21.91	5.69	9.40	157.2	3.63	14.96
128	*T. araraticum*	GAt	KU-8682	TR 61; 8682	IRQ	73.67	69.72	11.19	13.84	9.02	9.74	233.0	3.77	14.08
131	*T. araraticum*	GAt	PI 427357		IRQ	83.01	79.57	9.63	11.75	8.74	9.44	239.3	4.05	16.85
134	*T. aestivum* cv. Apostel	BAD	IG Pflanzenzucht		NA	46.55	48.20	16.07	18.58	5.89	8.75	147.0	5.05	23.45
139	*T. araraticum*	GAt	KU-8713	TR 69 TP072-TP076	IRQ	109.72	71.64	7.22	12.89	8.69	9.31	243.6	4.02	15.40
140	*T. araraticum*	GAt	KU-8945	TR 101-1	IRN	45.03	58.56	18.81	16.95	6.42	10.01	243.9	4.19	14.03
141	*T. araraticum*	GAt	IG 113297	2657	IRN	47.36	56.14	17.86	17.57	6.14	9.89	223.7	4.13	17.16
142	*T. araraticum*	GAt	KU-8925	1970-6-28-3-5-A-2; 2892	IRQ	70.09	68.34	11.51	13.86	6.61	9.51	253.1	3.89	15.21
143	*T. araraticum*	GAt	KU-8640	TR 56-1; 8640	IRQ	81.22	73.74	9.93	12.66	9.21	9.42	226.7	3.87	12.18
144	*T. araraticum*	GAt	KU-8918	2743; TR96	TUR	61.44	65.94	13.05	14.23	7.81	9.47	234.3	3.78	14.21
145	*T. dicoccon*	BA	PI 217637		IND	36.01	47.17	22.83	19.50	5.87	9.27	161.4	3.53	10.98
146	*T. aestivum* cv. Julius	BAD	KWS SAAT SE &Co.		NA	42.97	41.21	16.59	19.92	5.39	8.22	150.3	5.03	21.56
151	*T. araraticum*	GAt	KU-8593	TR 53; 8593	IRQ	64.08	51.87	12.95	18.72	6.63	9.76	245.4	4.07	14.92
153	*T. araraticum*	GAt	KU-8452	TR 46; 8452	IRQ	57.50	70.85	14.32	13.60	9.30	9.73	246.3	3.78	12.65
154	*T. araraticum*	GAt	TRI 16599	2399/3	ARM	64.08	52.62	13.05	18.79	7.95	9.84	218.8	4.04	12.97
155	*T. araraticum*	GAt	2005-7-5-9-4	3127	TUR	52.95	53.41	14.73	17.16	6.33	9.19	224.8	3.71	14.85

Geno-type no.	Taxon	Genome	Original sample number	Other identifiers	Origin	Mean Fe (mg/kg)	Mean Zn (mg/kg)	Mean Phyt:Fe	Mean Phyt:Zn	Mean P (mg/g)	Mean phytate (mg/g)	Mean GPC (g/kg)	TPC (µmol GAE/g)	ORAC (µmol TE/g)
156	*T. araraticum*	GAt	KU-8939	2753; TR99	TUR	65.93	47.86	11.75	18.82	6.42	9.08	195.5	3.90	13.27
158	*T. araraticum*	GAt	KU-8680	2845	IRQ	101.48	59.22	8.13	16.21	7.16	9.69	249.1	4.52	17.05
160	*T. aestivum*	BAD	CGN12270		IRN	50.84	41.00	14.10	20.29	6.22	8.40	150.7	4.54	20.00
161	*T. araraticum*	GAt	KU-8709	TR 67; 8709	IRQ	70.12	62.91	11.57	15.11	7.29	9.56	252.6	3.96	15.66
165	*T. dicoccon*	BA	CItr 14621		ETH	67.66	81.39	11.83	11.76	7.04	9.37	213.6	4.30	22.33
166	*T. urartu*	Au	PI 654310		TUR	86.16	49.79	8.89	17.88	7.09	8.98	211.6	4.53	17.91
167	*T. durum* cv. Wintergold	BA	SAATEN UNION		NA	25.65	42.52	30.10	21.43	6.15	9.11	150.0	4.39	24.04
169	*T. aestivum* cv. RGT Reform	BAD	RAGT Saaten		NA	64.84	51.87	10.84	15.95	6.09	8.31	174.4	4.74	21.29
174	*T. monococcum*	Ab	LPCH 95	ID 1737	MAR	54.83	38.03	14.22	23.76	6.38	9.10	148.4	4.43	16.17
177	*T. araraticum*	GAt	KU-8909	TR 93-2-3; 8909	TUR	79.20	69.05	10.28	13.79	6.83	9.60	230.8	5.98	13.27
180	*T. araraticum*	GAt	KU-8561	TR52; 8561	IRQ	65.06	68.18	12.53	13.91	6.95	9.57	235.7	5.40	10.63
181	*T. aestivum* cv. Nordkap	BAD	SAATEN UNION		NA	52.18	39.08	14.47	22.61	5.96	8.85	157.1	4.52	18.14
182	*T. monococcum*	Ab	PI 538722	ID 1260 SSD 2007+2009	TUR	70.07	65.16	11.23	15.08	6.09	9.19	182.9	4.80	13.93
185	*T. araraticum*	GAt	2005-7-5-9-4	3129	TUR	60.56	61.04	13.34	15.59	6.20	9.55	242.3	4.14	11.03
186	*T. araraticum*	GAt	PI 427371		IRN	75.01	65.64	10.75	14.47	6.52	9.51	248.5	4.55	12.48
189	*T. araraticum*	GAt	HTRI 11509/83	TR 133-1; 2776	IRN	65.49	67.30	12.15	13.83	6.55	9.39	253.3	5.31	16.93
192	*T. monococcum*	Ab	PI 191097	ID 1681	ESP	58.80	48.75	13.65	19.21	6.69	9.46	207.6	5.63	20.36

Geno-type no.	Taxon	Genome	Original sample number	Other identifiers	Origin	Mean Fe (mg/kg)	Mean Zn (mg/kg)	Mean Phyt:Fe	Mean Phyt:Zn	Mean P (mg/g)	Mean phytate (mg/g)	Mean GPC (g/kg)	TPC (µmol GAE/g)	ORAC (µmol TE/g)
193	*T. araraticum*	GAt	IG 117895	2681	SYR	50.99	52.00	16.03	18.25	6.18	9.56	239.5	4.91	14.95
194	*T. araraticum*	GAt	KU-8683	TR 62; 2717	IRQ	57.10	59.68	14.80	16.59	6.36	9.99	249.2	4.26	11.26
195	*T. monococcum*	Ab	G 863, PI 428151	MG 4278 ; ID 1331 EP030-EP035	ITA	44.36	44.43	17.96	20.35	5.42	9.09	164.1	4.93	19.51
198	*T. dicoccon*	BA	PI 352348		SYR	54.11	56.94	14.97	16.31	6.46	9.31	196.3	4.04	13.71
199	*T. araraticum*	GAt	KU-8944	2915	IRQ	84.39	60.47	9.38	15.31	6.67	9.34	235.2	4.10	14.53
200	*T. araraticum*	GAt	KU-1902		ARM	78.39	49.54	11.05	19.33	6.40	9.66	216.7	4.88	20.26
201	*T. boeoticum*	Ab	PI 427627	ID 758 EP079-EP086	TUR	76.29	27.02	10.76	35.48	8.67	9.68	242.5	4.07	19.41
202	*T. araraticum*	GAt	KU-1966	2704; TR44	TUR	69.89	59.58	12.21	16.77	6.45	10.09	216.4	3.91	14.42
203	*T. araraticum*	GAt	KU-1980A	1976-8-14-1-39; 2913	IRQ	85.16	82.84	9.72	11.70	7.22	9.78	248.6	4.34	17.50
205	*T. araraticum*	GAt	KU-8733	8733	IRQ	65.51	55.52	12.65	17.49	6.09	9.79	233.4	4.36	17.71
208	*T. dicoccon*	BA	PI 470739		TUR	49.02	45.65	15.22	19.11	5.89	8.80	160.3	4.14	18.37
210	*T. durum*	BA	DIC 193		ITA	32.05	57.68	24.27	15.69	6.08	9.10	164.6	4.29	22.87
215	*T. araraticum*	GAt	KU-1933	1976-7-27-2-yo; 2788	IRQ	73.88	55.70	11.50	17.81	6.55	10.01	263.4	4.57	16.70
217	*T. araraticum*	GAt	2006-6-20-11-6 D		TUR	67.37	45.16	12.10	21.07	5.98	9.59	236.6	NA	NA
218	*T. dicoccon*	BA	TRI 10324		SVK	53.23	44.11	14.80	20.76	6.30	9.24	173.6	4.31	17.35
219	*T. araraticum*	GAt	KU-8939	TR 99; 8939	TUR	62.27	48.69	13.99	22.19	6.24	10.28	220.9	4.03	16.86

Geno-type no.	Taxon	Genome	Original sample number	Other identifiers	Origin	Mean Fe (mg/kg)	Mean Zn (mg/kg)	Mean Phyt:Fe	Mean Phyt:Zn	Mean P (mg/g)	Mean phytate (mg/g)	Mean GPC (g/kg)	TPC (µmol GAE/g)	ORAC (µmol TE/g)
221	*T. araraticum*	GAt	KU-8866	1970-6-17-1-4-8-2; 2873	IRQ	80.11	64.76	9.68	14.12	6.61	9.08	261.1	4.57	15.73
222	*T. araraticum*	GAt	KU-8545	TR 51;8545	IRQ	75.92	47.91	10.66	19.76	6.96	9.56	239.8	4.47	21.91
231	*T. araraticum*	GAt	KU-1960	2794	TUR	73.13	53.20	10.81	17.42	6.29	9.33	230.4	4.14	17.06
232	*T. araraticum*	GAt	KU-8938	TR 98-1-1; 2749	TUR	59.14	38.51	12.85	22.76	6.17	8.64	205.8	4.20	20.29
233	*T. araraticum*	GAt	PI 596290		TUR	61.78	46.25	12.51	20.17	6.37	9.09	229.5	4.34	16.20
238	*T. araraticum*	GAt	KU-8529	8529	IRQ	70.38	58.75	11.57	16.08	6.66	9.53	226.8	4.59	18.93
242	*T. dicoccoides*	BA	KU-14474		ISR	51.78	27.04	14.78	33.14	5.14	9.05	227.8	4.53	16.37
243	*T. dicoccon*	BA	IG 45091		OMN	66.93	48.89	11.73	18.87	5.94	9.28	160.9	4.06	22.81
244	*T. araraticum*	GAt	IG 119456		SYR	64.80	58.31	13.55	17.63	6.65	10.37	261.4	4.47	15.21
246	*T. araraticum*	GAt	TRI 11345/80	TR 120; 2763	IRQ	73.62	78.24	11.55	12.74	7.02	10.05	252.0	4.50	17.33
248	*T. dicoccoides*	BA	KU-8812		IRQ	60.13	40.39	13.00	25.77	6.83	9.21	230.2	5.10	16.61

Notes: Fe=iron; P=phosphorus; Zn=zinc; T=Triticum; GPC=grain protein content; TPC=total phenolic content; ORAC=oxygen radical absorbance capacity; AFG=Afghanistan; ARM=Armenia; AZJ=Azerbaijan; ESP=Spain; ETH=Ethiopia; FRA=France; GEO=Georgia; GRC=Greece; HUN=Hungary; IND=India; IRN=Iran; IRQ=Iraq; ISR=Israel; ITA=Italy LBN=Lebanon; MAR=Marocco; OMN=Oman; ROM=Romania; RUS=Russia; SU= former Soviet Union; SVK=Slovakia; SYR=Syria; TUR=Turkey; NA=Not available.

Table SI 3: Grain quality parameters for each taxon (mean values)

Taxon	**Mean Fe (mg/kg)**	**SD Fe**	**VarCoeff Fe (%)**	**Mean Zn (mg/kg)**	**SD Zn**	**VarCoeff Zn (%)**	**Mean Phyt:Fe**	**SD Phyt:Fe**	**VarCoeff Phyt:Fe (%)**	**Mean Phyt:Zn**	**SD Phyt:Zn**	**VarCoeff Phyt:Zn (%)**
T. aestivum	42.84	9.13	21.30	48.68	13.86	28.48	18.63	4.67	25.09	19.33	3.92	20.28
T. aestivum cv.	51.63	9.71	18.80	45.09	7.64	16.94	14.49	2.76	19.03	19.27	3.24	16.79
T. araraticum	72.52	16.74	23.08	60.12	12.18	20.26	11.73	2.86	24.36	16.34	3.19	19.52
T. boeoticum	79.33	8.82	11.11	57.62	17.55	30.46	10.26	1.22	11.87	18.51	7.88	42.57
T. dicoccoides	60.73	12.44	20.48	53.02	16.77	31.62	13.65	2.77	20.30	19.78	7.40	37.39
T. dicoccon	55.68	14.19	25.48	53.98	14.84	27.49	14.79	3.63	24.51	17.75	3.29	18.52
T. durum	42.99	11.17	25.98	59.45	3.29	5.53	19.80	4.81	24.30	15.76	0.93	5.90
T. durum cv.	36.45	11.79	32.35	42.95	5.80	13.50	23.25	7.00	30.10	21.25	2.50	11.79
T. monococcum	53.29	12.24	22.97	47.26	12.63	26.73	14.82	3.78	25.53	19.53	4.32	22.13
T. timopheevii	57.63	8.73	15.14	49.82	5.83	11.71	11.97	2.13	17.82	16.07	2.48	15.41
T. urartu	75.81	10.68	14.08	53.93	11.28	20.91	10.62	1.88	17.67	17.73	3.38	19.10
T. zhukovskyi	73.33	12.72	17.35	109.62	18.02	16.44	11.08	3.03	27.34	8.63	2.23	25.80
Biological status												
Landrace	53.94	14.33	26.57	55.36	19.98	36.09	15.12	4.40	29.11	17.46	4.34	24.83
Modern cultivar	46.57	12.66	27.19	44.38	7.15	16.11	17.41	6.20	35.62	19.93	3.15	15.82
Wild	72.11	16.18	22.45	58.97	13.25	22.47	11.74	2.82	24.02	16.87	4.35	25.80

Taxon	**Mean P (mg/g)**	**SD P**	**VarCoeff P (%)**	**Mean Phytate (mg/g)**	**SD Phytate**	**VarCoeff Phytate (%)**	**Mean GPC (g/kg)**	**SD GPC**	**VarCoeff GPC (%)**
T. aestivum	6.14	0.63	10.33	8.97	0.63	7.02	163.0	24.42	14.98
T. aestivum cv.	5.83	0.28	4.86	8.53	0.30	3.48	157.2	12.24	7.79
T. araraticum	6.90	0.96	13.89	9.55	0.49	5.13	235.6	19.48	8.27
T. boeoticum	7.41	0.96	12.91	9.50	0.35	3.67	216.1	23.87	11.05
T. dicoccoides	6.33	0.81	12.80	9.42	0.54	5.74	216.5	31.74	14.66
T. dicoccon	6.30	0.50	7.94	9.20	0.44	4.74	179.9	25.16	13.98
T. durum	6.40	0.37	5.80	9.44	0.37	3.90	166.4	8.41	5.05
T. durum cv.	6.14	0.25	4.03	9.07	0.20	2.20	153.4	13.17	8.59
T. monococcum	6.26	0.51	8.16	8.82	0.73	8.31	172.7	18.84	10.91
T. timopheevii	6.10	0.46	7.51	7.99	0.99	12.35	170.2	7.51	4.41
T. urartu	7.03	0.83	11.79	9.28	0.29	3.07	212.5	15.30	7.20
T. zhukovskyi	8.96	1.76	19.59	9.15	0.75	8.15	238.6	15.52	6.50
Biological status									
Landrace	6.43	0.96	14.85	8.97	0.73	8.17	178.0	27.20	15.28
Modern cultivar	5.94	0.31	5.19	8.71	0.37	4.24	155.9	12.69	8.14
Wild	6.89	0.96	14.00	9.52	0.48	5.06	231.2	22.58	9.77

Taxon	**Mean TPC (µmol GAE/g)**	**SD TPC**	**VarCoeff TPC (%)**	**Mean ORAC (µmol TE/g)**	**SD ORAC**	**VarCoeff (%)**
T. aestivum	4.54	0.49	10.70	16.08	4.57	28.42
T. aestivum cv.	4.83	0.39	8.12	21.11	3.24	15.35
T. araraticum	4.33	0.50	11.52	15.78	4.00	25.34
T. boeoticum	4.75	0.44	9.26	17.59	4.15	23.61
T. dicoccoides	4.41	0.46	10.37	17.87	3.59	20.10
T. dicoccon	4.13	0.40	9.80	17.32	4.73	27.33
T. durum	4.04	0.35	8.72	17.97	5.62	31.26
T. durum cv.	4.54	0.52	11.54	19.71	5.17	26.25
T. monococcum	4.83	0.63	12.96	17.40	3.05	17.52
T. timopheevii	3.79	0.13	3.48	13.40	4.92	36.71
T. urartu	4.87	0.42	8.69	20.53	5.03	24.50
T. zhukovskyi	6.32	1.01	15.91	25.79	2.73	10.58
Biological status						
Landrace	4.46	0.79	17.82	17.39	5.05	29.03
Modern cultivar	4.74	0.46	9.76	20.64	4.04	19.59
Wild	4.40	0.51	11.65	16.36	4.23	25.84

Notes: Fe=iron; P=phosphorus; Zn= zinc; *T*=*Triticum*; GPC=grain protein content; ORAC=oxygen radical absorbance capacity; TPC=total phenolic content; VarCoeff=Coefficient of Variation; SD=standard deviation

Acknowledgments

I would like to express my sincere gratitude to Prof. Dr Michael Frei for giving me the chance to do my PhD thesis in his institute and who guided me through my scientific journey. His continuous support and the freedom he gave me to conduct my research supported me in exploring the topic and solving difficult stages.

My sincere thanks goes to my second supervisor Prof. Dr. Agnieszka Golicz for her suggestions and valuable conversation throughout my PhD.

I also sincerely thank Dr. Benjamin Kilian for his continuous support during my work and for sharing his vast knowledge on wheat wild relatives. In that regard I would also like to thank the BOLD Project.

My sincere thanks goes to Prof. Dr. Hakan Özkan for providing scientific support during my research and for hosting me during my research stay at the University of Çukurova, Adana in January 2024. In that regard I would also like to thank the DAAD for the funding and the opportunity to do research at the University of Çukurova

I am very grateful for my colleagues at the Department for Agronomy and Crop Physiology. I thank Liane Renno for her constant support during field work, harvest and lab work. Sincere gratitude goes to Erika Schick for her assistance during harvest and lab work. Further I would like to thank Sumitra Pantha for exploring the wheat wild relatives with me. My sincere gratitude goes to Markus Kolmer, who supported me on the field and contributed with valuables ideas to enable the cultivation of wild wheats in Giessen. Moreover I would like to thank Yavar Vaziritabar, Denise Schudt, Zain Ali, Bariş Çankaya, Maria Changezi, Sophia Bohländer, Alena Zink, Miquel Jorque Mateo, Christine Kessler-Turturici, Muhammad Shahedul Alam, Dr. Tabea Selzer, Dr. Feng Yan and Dr. Johanna Krippner.

I am especially thankful for my husband Kevin, my parents and my brother Max for their continuous support and encouragement.

I would like to pay my special regards to the Studienstiftung des deutschen Volkes for providing the opportunity to conduct my PhD and for offering a diverse range of activities and programs that helped me to develop myself further.

Eidesstaatliche Erklärung

Erklärung gemäß der Promotionsordnung des Fachbereichs 09 vom 07. Juli 2004 § 17 (2)

„Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe.

Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.

Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.

Giessen, _________________ ___________________________

Frederike Zeibig