


# The role of omics in improving the orphan crop tef

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## Review

## The role of omics in improving the orphan crop tef

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**Tef or teff [*Eragrostis tef* (Zucc.) Trotter] is a cereal crop indigenous to the Horn of Africa, where it is a staple food for a large population. The popularity of tef arises from its resilience to environmental stresses and its nutritional value. For many years, tef has been considered an orphan crop, but recent research initiatives from across the globe are helping to unravel its undisclosed potential. Advanced omics tools and techniques have been directed toward the exploration of tef's diversity with the aim of increasing its productivity. In this review, we report on the most recent advances in tef omics that brought the crop into the spotlight of international research.**

### Role of tef in food and nutrition security

Food and nutrition insecurity are major challenges in the developing world. These issues are exacerbated by the fact that a large proportion of arable land in emerging countries is severely affected by the climate crisis. Although major cereal crop species such as rice, wheat, and maize contribute significantly to global food security, indigenous crops are essential as they are adapted to the local environment and user needs and play a vital role in the diet of the population of emerging countries. These crops are commonly known as orphan or underutilized crops due to the little scientific attention that they have received.

Tef or teff [*Eragrostis tef* (Zucc.) Trotter], a cereal crop from the Horn of Africa, is considered an orphan crop. It is an important indigenous crop, especially in Ethiopia, where it is annually cultivated on over 3 million hectares of land, equivalent to 30% of the total area allocated to cereals [1]. Tef is estimated to be a staple food for over 70 million people in Ethiopia alone. While the grain of tef is a valuable food source for humans, the straw is the most palatable and preferred feed for livestock [2]. Tef is considered a major cash crop, since its grain and straw fetch a higher price than that of wheat and barley.

In addition to its high content of iron, calcium, and other nutrients, the grain of tef is gluten-free [3], which makes it an alternative food for people with celiac disease. The low glycemic index (GI) [4] and high iron content, as well as a high amount of lysine, a major limiting amino acid in cereals [5,6], have resulted in tef being heralded as a global 'superfood' [7,8].

### Resilience of tef to extreme environmental conditions

Tef adapts to a wide variety of environmental conditions, including extreme moisture and temperature. These areas range from warm to cold and from semiarid to humid. In terms of elevation, tef is suited to areas in Ethiopia ranging from 750 m a.s.l. to 2500 m a.s.l. Compared with other cereals, tef adapts well to water scarcity [9]. In addition to adapting to low soil moisture, tef escapes terminal drought, which occurs late in the cropping season due to its short life cycle.

### Highlights

Tef research has little benefited from omics due to neglect of the crop by the global scientific community. However, the few utilized tools showed promising results.

Chromosome-scale assembly of the genome reveals the evolution of tef. The whole-genome sequencing of drought-resurrecting *Eragrostis nindensis* and desiccation-sensitive *Eragrostis curvula* shows genes that play key roles in drought responses.

A few studies using omics tools have revealed differentially expressed genes, proteins, and metabolites in tef plants exposed to drought.

Molecular markers have deciphered diversity in tef germplasm collected from diverse agroecologies in Ethiopia.

The application of targeting induced local lesions in genomes resulted in mutant tef lines with a semidwarf stature, which were later bred to locally adapted and high-yielding varieties.

Genome editing using 'Green Revolution' genes resulted in a semidwarf and lodging-tolerant tef plant.

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Tef is also tolerant to waterlogging, especially during germination and early crop establishment due to elevated amounts of aerenchyma tissue in the root [10]. Due to this enhanced performance under waterlogged soils, tef is unique, as it is also the farmers' crop of choice in the highlands of Ethiopia that are dominated by poorly drained vertisols.

### Challenges to tef production

Tef is popular with both growers and consumers; however, its productivity is low compared with that of the most common cereals, wheat, maize, and rice. The major constraint affecting the productivity of tef is lodging, the permanent displacement of the stalk from the upright position. The primary cause of lodging in tef is the weak stalk, which easily succumbs to wind, rain, and nitrogenous fertilizer. Although tef performs better than other cereals under drought and waterlogging, it is significantly affected by heat, soil acidity, and salinity, which are prevalent in tef-growing areas in Ethiopia. Soil salinity affects the germination of tef seeds [11]. Prolonged drought period affects the development of tef particularly when the stress occurs during the flowering and grain-filling stages, since moisture deficit affects the translocation of carbohydrates from the source to the sink [12]. Among biotic constraints, weeds are the major bottleneck in tef husbandry as tef is a poor competitor to weeds. The low productivity of tef at the national level in Ethiopia is also related to the low dissemination of inputs such as improved seeds, fertilizers, and herbicides to over 6.6 million tef farmers in the country [1,13].

### Exploiting diversity to improve productivity

For decades, the National Tef Improvement Program in Ethiopia has focused on boosting productivity by developing and disseminating new tef varieties with enhanced performance. This has been done through rigorous selection from the biodiversity [14] found in an extensive collection of tef germplasm maintained at the Ethiopian Biodiversity Institute (EBI).

Huge diversity is present in tef germplasm in panicle color (Figure 1A), panicle shape (Figure 1B), and traits such as flowering and maturity time and grain yield (Figure 1C). Candidate lines with desirable traits are hybridized with existing lines and the resulting hybrids are then screened for the trait(s) of interest (Box 1).

Based on the concerted efforts of the Ethiopian federal and regional research institutions, 58 tef varieties have been released for use by the farming communities. Improved agronomic technologies have also been developed to create optimum conditions for tef husbandry. However, the dissemination of technologies has reached only a fraction of the roughly 6.6 million farmers annually cultivating tef in Ethiopia. Due to this, the national average grain yield of tef is only  $1.8 \text{ t ha}^{-1}$  [1], although the potential yield can reach up to  $4 \text{ t ha}^{-1}$ .

Tef research has also been advancing through international partnerships, which include the application of molecular markers and molecular breeding techniques to assess and exploit tef diversity [15–19], whole-genome sequencing [20,21], and the targeting of the gene(s) responsible for semidwarfism and lodging tolerance using genome editing methods [22].

In addition to elucidating genes responsible for traits of importance in tef husbandry [10,23,24], the partnership between the University of Bern in Switzerland and the Ethiopian Institute of Agricultural Research (EIAR) released four high-yielding and drought-tolerant tef varieties that are popular among farmers [25–27]. These partnerships have contributed significantly to scientific knowledge and the boosting of tef productivity.

This review focuses on progress made in tef in **genomics** (see **Glossary**) and related omics tools, which include **transcriptomics**, **proteomics**, and **metabolomics**. In addition, it discusses the

### Glossary

**Allotetraploid:** an organism with four sets of chromosomes resulting from the hybridization of two different species.

**Comparative genomics:** systematic study of the similarities and differences in gene content and structure of whole genomes.

**DNA binding with one finger (DOF):** a group of plant-specific TFs that play important roles in plant growth, development, and response to biotic and abiotic stresses.

**Epigenomics:** the study of how cells control gene activity without changing the DNA sequence.

**Functional genomics:** the study of how genes and intergenic regions of the genome contribute to different biological processes.

**Gene Ontology (GO) term:** a short phrase that is typically used to represent the individual components of ontologies, which unify the representation of gene and gene product attributes across all species.

**Genome size:** the total amount of DNA contained in one copy of a single complete genome.

**Genome-wide association study (GWAS):** identifies associations between genetic markers called SNPs and a phenotype of interest and traits.

**Genomics:** a field of biology focusing on the structure, function, evolution, mapping, and editing of genomes.

**Homoeologous chromosomes:** duplicated chromosomes that are derived from different parental species and are related by ancestry.

**Ionomics:** the study of the complete mineral nutrient and trace elemental composition of a plant species.

**Metabolomics:** the identification and quantification of small molecules present in biological systems.

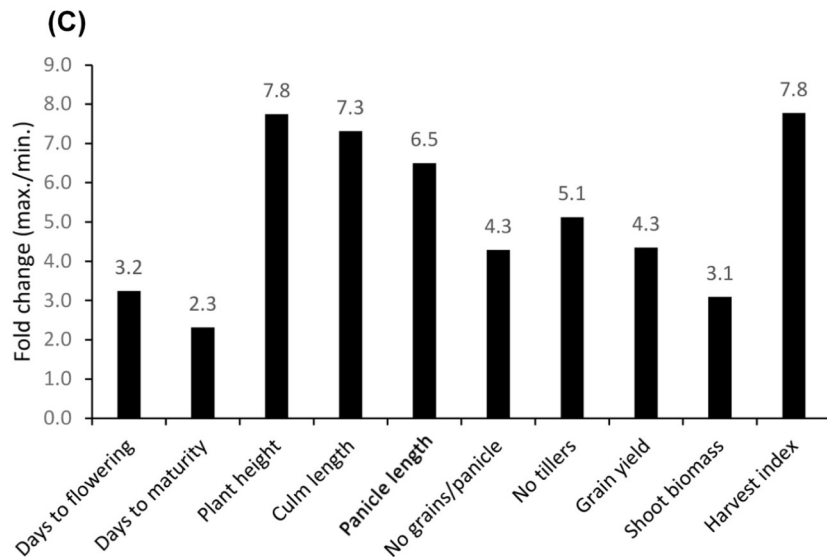
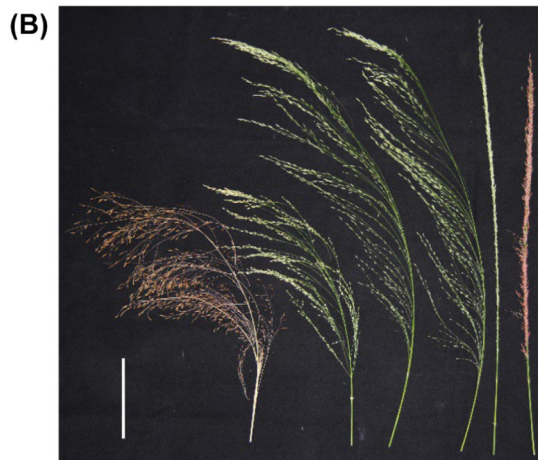
**Metagenomics:** the study of environmental microbial communities using a suite of genomic tools to directly access their genetic content.

**miRNA:** small, single-stranded, noncoding RNA molecules containing 21–23 nucleotides.

**Multomics:** integrating the analysis of multiple omics datasets.

**Orthologous genes:** genes that evolve from a common ancestral gene by speciation and have usually retained a similar function in different species.

**Pangenome:** a collection of all DNA sequences of a species that contains sequences shared by all individuals (core



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(See figure legend at the bottom of the next page.)

genome) and is also able to display sequence information unique to each individual (variable genome).

**Phenomics:** the study of an overall organism and how the characteristics or traits of an organism that we can see (its phenotype) fits with the information we know about its genes (genomics) and proteins (proteomics).

**Phylogenetic tree:** a diagram that represents evolutionary relationships among organisms.

**Plastome:** the genome of a plastid, which is extensively used in phylogenetic analyses.

**Proteomics:** a study of protein properties to obtain a global view of cellular processes at the protein level.

**Quantitative trait loci (QTLs):** a region of DNA associated with a specific phenotype or trait that varies within a population.

**Recombinant inbred line (RIL):** an organism with chromosomes that incorporate an essentially permanent set of recombination events between chromosomes inherited from two or more inbred strains.

**Retrotransposable element:** a type of genetic component that copies and pastes itself into various genomic locations by converting RNA back into DNA.

**Simple sequence repeats (SSRs):** synonym, microsatellites; variable numbers of tandem repeats that constitute genomic repetitive regions.

**Single-cell RNA-seq (scRNA-seq):** set of methods for the sequencing of transcriptomes of single cells.

**Targeting induced local lesions in genomes (TILLING):** a reverse-genetics approach where traditional chemical mutagenesis is followed by high-throughput to facilitate screening for point mutations.

**Transcription factors (TFs):** proteins that can bind to specific DNA sequences to control transcription.

**Transcriptomics:** the study of the complete set of RNA transcripts, both coding and noncoding, expressed in a given entity such as a cell, tissue, or organism.

**Vascular plant one-zinc finger protein (VOZ):** plant-specific TF family.  
**WRKY:** TF that binds DNA.

contributions of **functional genomics** and reverse-genetics approaches such as **targeting induced local lesions in genomes (TILLING)** in tef variety development and the further boosting of productivity in farmers' fields.

### Genomics studies in tef

#### Evolution and genome size of tef

Tef belongs to the genus *Eragrostis* (family Poaceae), which has about 350 species, of which tef is the only species cultivated for human consumption. It is an **allotetraploid** ( $2n = 4x = 40$ ), and earlier studies based on molecular evidence showed that *Eragrostis pilosa* might be the ancestor of tef [28]. The fact that the intercross between the two species produces viable offspring reinforces this interpretation. *E. pilosa* also shares remarkable morphological similarities with tef, except for its spikelet shattering trait, which may have been lost through domestication [29]. However, since *E. pilosa* is also an allotetraploid, the true diploid progenitors of tef have yet to be determined. A chromosome-scale assembly of the *Dabbi* cultivar showed that the two genomes of tef diverged approximately 5 million years ago (Mya) [21]. A recent genotyping by sequencing (GBS) study, which included potential progenitors, allowed the construction of a **phylogenetic tree** based on the waxy gene, suggesting that *Eragrostis aethiopica* may be the diploid progenitor of subgenome A and *Eragrostis heteromera* may be the closest relative to subgenome B [15]. Nonetheless, the question regarding an intermediate tetraploid progenitor resulting from the hybridization of the two diploid progenitors remains open.

The **genome size** of tef has been determined by several independent researchers using flow cytometry. Although huge diversity exists in tef germplasm in terms of panicle shape and color and several agronomic traits [18] (Figure 1), at least four studies indicated that variations in genome size among landraces and improved varieties were insignificant [30–33]. This might be due to the limited number of genotypes used for these studies.

#### Chloroplast genome

Information about the chloroplast genome is useful in molecular marker development and biogeographic studies. Due to high conservation, chloroplast genomes (also known as **plastomes**) are ideal for phylogenetic analyses. The recent study on 32 tef genotypes indicated that the size of plastomes ranged from 134 349 to 134 437 bp [34]. Genome annotation on the tef plastome revealed 112 individual genes, including 77 protein-coding, 31 tRNA, and four rRNA genes. In general, this study showed that the degree of intraspecific sequence variation was low. Another study indicated that some wild *Eragrostis* species, particularly *E. pilosa*, possess plastome sizes close to that of tef, indicating close relationships with tef [35]. Interestingly, the plastome of finger millet [*Eleusine coracana* (L.) Gaertn.], a species in the same subfamily (Chloridoideae) as tef, differs only slightly in size from that of tef [36] indicating conservation of the chloroplast genome.

#### Whole-genome sequencing

Tef entered the genomic era in 2014 when the first whole-genome sequence of the improved variety *Tsedey* (also known as DZ-Cr-37) was produced [20]. The draft sequence comprised 672 Mbp, representing 87% of the tef genome size. Approximately 42 000 **orthologous genes** to those in sorghum and rice were identified and annotated. The draft genome was used to identify novel **simple sequence repeat (SSR)** markers, to investigate target genes for

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Figure 1. The collection of tef germplasm contains a vast diversity. (A) Panicle color in the field in Ethiopia. Photograph: Z. Tadele. (B) Panicle shape ranging from very loose to very compact. Photograph: R. Schneider. (C) Fold changes between the minimum and maximum values in key agronomic traits. Adapted from [11].



### Box 1. Unique properties of tef

Tef [*Eragrostis tef* (Zucc.) Trotter] is among the most important cereal crops in the Horn of Africa, especially in Ethiopia where it is annually cultivated on over 3 million hectares of land, equivalent to 30% of the total area allocated to cereals [1]. Some characteristics that make tef a good model crop are:

- Its high drought tolerance [9], while maize, rice, wheat, and barley are sensitive to moisture scarcity.
- Its tetraploid genome. Except for wheat with tetraploid and hexaploid genomes, most cereals, including barley, rice, sorghum, and maize, are diploid.
- Its C4 photosynthesis system. While barley, rice, and wheat are C3 plants, sorghum and maize are C4. C3 and C4 plants respond antagonistically to water stress.
- Its gluten-free grain [3] makes it an alternative food for people with celiac disease. Its low glycemic index (GI) [4] and high amounts of the amino acid lysine, a major limiting amino acid in cereals, as well as a high iron content [5], being rich in ascorbic acid, and having high antioxidant activities, makes tef among the most attractive cereals. Due to lack of gluten and low GI, tef has been heralded as a global 'superfood' or 'super grain' [7].
- Its short maturity period, which takes only 3 months from sowing to harvesting (while all major cereal crops mature in a minimum of 4 months).
- Its tolerance to early waterlogging [10], unlike wheat, maize, and barley. Tef can rapidly germinate and establish well when sown on completely waterlogged fields. Due to this enhanced performance under flooded soils, tef is unique. In the highlands of Ethiopia dominated by poorly drained vertisols, tef is the farmers' crop of choice.

abiotic stress resistance studies, and to understand the evolution of prolamin family proteins responsible for the immune response to gluten [20].

The second chromosome-scale assembly of the whole-genome sequence of tef focused on the cultivar *Dabbi* and identified two complete sets of **homoeologous chromosomes** with most genes maintained as syntenic gene pairs [21]. The same authors estimated that the tef polyploidy event occurred around 1.1 Mya and that the two subgenomes diverged around 5 Mya. Interestingly, no large-scale structural rearrangements, homoeologous exchanges, or biased gene losses were detected in tef [21], unlike other allopolyploid species.

Whole-genome sequencing has also been conducted on related species in the *Eragrostis* genus, focusing on contrasting responses to water regimes. Sequences are available for the drought-resurrecting *Eragrostis nindensis* and the desiccation-sensitive *Eragrostis curvula* [37]. By comparing the differences in the genomic regions of *E. nindensis* and tef, changes in chromatin architecture, methylation, gene duplication, and expression dynamics related to desiccation were identified [38]. In addition, a small set of seed-related orthologs were found to have expression patterns specific to desiccation-tolerant species. The *de novo* genome of *E. curvula* was obtained by sequencing 602 Mb of a diploid genotype using a strategy that combined long-read length sequencing with chromosome conformation capture [39]. Repetitive sequences comprise a large proportion of the DNA in eukaryotic genomes and significantly affect gene regulation. A study in the tef *Enatite* genotype identified 1389 medium/highly repetitive sequences that collectively represent about 27% of the genome [40]. This value is not high compared with other cereals, particularly maize, where **retrotransposable elements** alone comprise more than 50% of the genome [41], and is in line with the relatively small tef genome. In general, information from whole-genome sequencing of tef and related species may accelerate the identification and mapping of polymorphism markers, facilitate **genome-wide association studies (GWASs)** and simplify **quantitative trait locus (QTL)** identification for valuable traits. Moreover, possession of the entire genomic sequence enables the generation of genome-specific oligo primers, which can be used in the high-throughput TILLING technique of crop improvement so that candidate tef lines harboring traits of interest can be developed.

### Comparative genomics

**Comparative genomics** refers to the study of genomes of different species to identify their evolutionary and molecular relationships. The phylogenetic tree in [Figure 2](#) shows the relationship

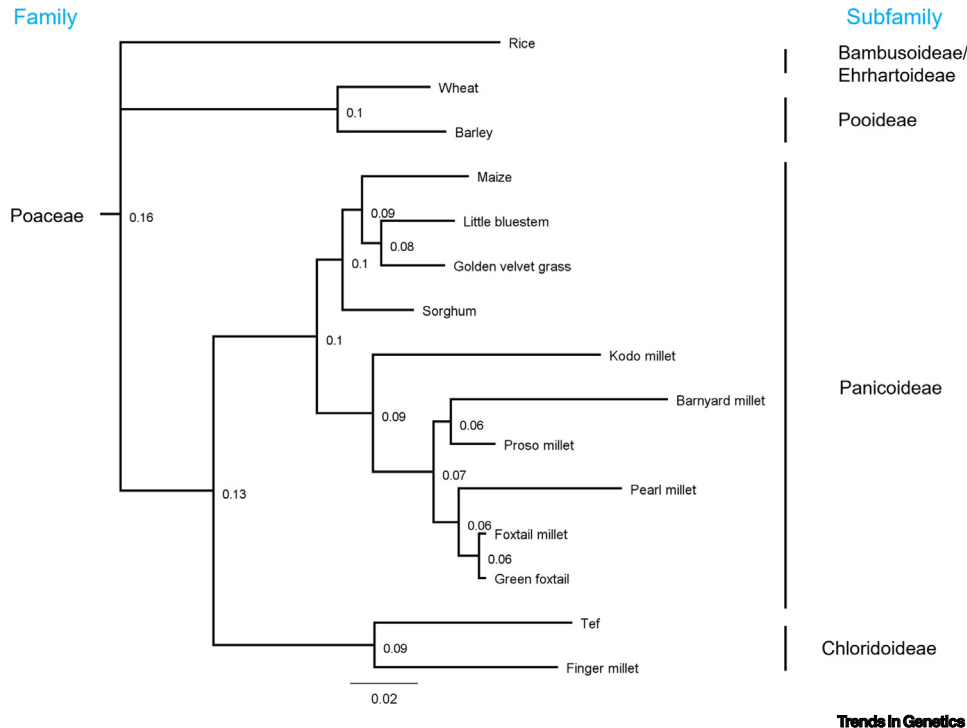


Figure 2. Phylogenetic tree for selected plants from the grass family including tef (*Eragrostis tef*) using partial sequences of the WAXY gene from rice (*Oryza sativa*, FJ235770.1), bread wheat (*Triticum aestivum*, KF861808), barley (*Hordeum vulgare*, X07931), maize (*Zea mays*, EU041692), little bluestem (*Cleistachne sorghoides*, AF446081.1), golden velvet millet (*Eulalia villosa*, JN560727.1), sorghum (*Sorghum bicolor*, EF089839), kodo millet (*Paspalum simplex*, AF318770.1), barnyard millet (*Echinochloa frumentacea*, FR845943.1), proso millet (*Panicum millaceum*, GU199268), pearl millet (*Pennisetum glaucum*, AF488414), foxtail millet (*Setaria italica*, AB089143), green foxtail (*Setaria viridis*, XM\_034735693.1), tef (*Eragrostis tef*, AY136939), and finger millet (*Eleusine coracana*, AY508652) obtained from the NCBI database. The maximum likelihood tree was inferred using MEGA 11 and the default model of HKY85 + G. The scale bar reflects evolutionary distance, measured in units of substitution per nucleotide site.

of tef to other grass family members [20]. Tef and finger millet [*E. coracana* (L.) Gaertn.] are closest; both belong to the subfamily Chloridoideae. Hence, tef can also benefit from the recent whole-genome sequencing of finger millet [42], which reveals chromosome-level relationships among finger millet, tef, and other closely related species. A recent review indicated that the completion of the whole-genome sequence for 12 orphan crops, including tef, provides significant advances in crop improvement [43].

Using information from the Green Revolution dwarfing genes from rice (*sd1*) and wheat (*rht1*), ortholog genes were cloned and studied in 31 tef accessions [44]. *In silico* studies based on other advanced systems have enabled researchers to clone tef genes related to drought tolerance [45] and three **transcription factors (TFs)** – namely, **WRKY**, **vascular plant one-zinc finger protein (VOZ)**, and **DNA-binding with one finger (DOF)** – which are related to drought tolerance [46–48].

### Functional genomics

Functional genomics has applications in genomics-assisted breeding of crops. In this case, molecular markers are used to construct genetic maps that identify QTLs associated with specific

trait(s) of interest. Although several attempts were made to develop genetic maps for tef, the latest genetic map was created using 116 **recombinant inbred lines (RILs)** derived from an interspecific cross between tef accession *Kaye Murri* and wild *E. pilosa* [49]. The study, which investigated the relative efficiencies of different molecular markers, indicated that 37.6% of the markers resulted in scoreable polymorphism in tef [50].

By contrast, GBS was implemented on 40 *Eragrostis* species and 42 tef lines to investigate SNPs [15]. Mapping individual reads to the tef reference genome revealed that *E. pilosa*, *E. aethiopica*, *Eragrostis obtusa*, *Eragrostis ferruginea*, *Eragrostis lugens*, and *Eragrostis lehmanniana* had 92% of their sequences represented in the tef reference genome, indicating that these species are the most closely related to tef [15] of the ones tested. Similarly, restriction site-associated DNA (RAD) sequencing using 43 tef landraces, one mutant line, and two wild *Eragrostis* species indicated higher nucleotide diversity in the two wild *Eragrostis* species than in the landraces, which were all grouped into one cluster [16]. Hence, they can be utilized in future tef improvement programs. Recently, a diversity study using 366 representative tef germplasms identified ten genetic clusters associated with climate variation in Ethiopian geography, phenotypic traits, and climate variation on the landscape [19].

Additionally, tef researchers have been able to identify genes involved in key agronomic traits. For instance, a candidate gene approach was applied to pinpoint the  $\alpha$ -tubulin 1 gene, which affects microtubules, producing a semidwarf phenotype in the *kegne* tef mutant line [24].

Genomics has also been effectively utilized in high-throughput techniques, particularly in TILLING. TILLING is a reverse-genetic technique that uses traditional random mutagenesis mediated by a mutagen followed by the identification of mutations in a specific target gene [51]. It has been effectively applied in diverse crops to identify mutant lines harboring traits of interest. The advantage of TILLING is that the mutants are not considered transgenic. Hence, they can be integrated into breeding programs without the lengthy and expensive process required by biosafety regulations. Tef TILLING was performed at least by two groups. The first group mutagenized four improved tef varieties using ethyl methanesulfonate (EMS), a chemical known to introduce point mutations in the genome, followed by screening for key agronomic traits, particularly for semidwarfism using a LiCor sequencer after cleaving PCR products with endonuclease CEL I [52]. A number of candidate semidwarf and lodging-tolerant lines were sent to the EIAR, where they were integrated into the breeding program through crossing to locally adapted varieties. The second tef TILLING platform implemented next-generation sequencing to screen 21 000 EMS-mutagenized tef lines using homeologs of *dw3* of sorghum and *rht1* of wheat [53]. According to the authors, six independent mutations were validated by Sanger sequencing, indicating that high-throughput sequencing can effectively identify potentially valuable mutations in tef.

The first successful genome editing using CRISPR/cas9 targeted an ortholog of the rice SEMIDWARF-1 (SD-1) gene and resulted in semidwarf and lodging-resistant tef plants [22]. Genome editing is facilitated by the efficiency of regeneration and transformation of the plant. Although both regeneration and transformation protocols were earlier established for tef [54], more efficient methods are required to fully exploit the benefit of genome editing in tef improvement.

### Transcriptomics in tef

Transcriptomics refers to the study of an entire collection of RNA transcripts from an organism allowing the identification of differentially expressed genes in a tissue-specific manner or an understanding of the transcriptional response under specific environmental conditions [55]. The



first tef transcriptome sequencing resulted in the identification of approximately 38 000 transcripts with 33 000 annotated genes [20].

A transcriptomic study showed that the tef *Tsedey* genotype tolerates waterlogging by forming additional adventitious roots and aerenchyma structure [10], similar to rice [56]. RNA-seq analysis using *Tsedey* showed that 37 genes were upregulated while 19 were downregulated under waterlogging conditions [10]. Among upregulated genes, those in the cell wall, transport, protein regulation, carbohydrate metabolism, and secondary metabolism were the major categories. Cell wall-related genes, *B-expansins* and *xyloglucan*, were upregulated in *Tsedey* under excessive-moisture conditions [10].

TFs are genes that regulate transcription through binding to specific sequences or by protein–protein interaction. An *in silico* study using information from sorghum, rice, and other species enabled tef researchers to identify 33 DOF TFs in tef [46]. Dof domain proteins are plant-specific TFs with a highly conserved DNA-binding domain involved in photosynthesis as well as biotic and abiotic stresses.

A recent *in silico*-based functional genomic analysis using the Drought Stress Gene Database [57] and CrealESTDb [58] identified 729 drought-responsive genes in tef and validated their expression using quantitative RT-PCR [45]. These genes are involved in diverse plant functions.

It is noteworthy that plant transcriptomic responses to environmental cues or nutrient availability can be assessed not only at a tissue-specific but also at the single-cell level using **single-cell RNA-seq (scrRNA-seq)** technology [59]. Using this method, candidate genes involved in maize ear traits were discovered with a potential to boost productivity [60].

### Epigenomics in tef

**Epigenomics** studies heritable changes in gene expression activity caused by factors other than DNA substitution, such as histone modification, DNA methylation, and the action of noncoding RNA. A study in tef indicated that **miRNAs** are expressed more in the drought-tolerant genotype *Tsedey* than in the susceptible *Alba* in both shoots and roots [61]. Some of these miRNAs, such as miR395, miR396, miR398, and miR399, were also negatively expressed in barley and tobacco [61], suggesting a conserved biological function among species. The targets of the above tef miRNAs include calcium-dependent protein kinases (*CDPKs*), TFs, and transporters. In agreement with this, the expression of *CDPKs* was also elevated in arabidopsis (*Arabidopsis thaliana*) under cold, salt, and drought [62]. Similarly, overexpression of *OsCDPK7* improved cold and salt tolerance in rice [63].

Future research using tef genotypes with differential tolerance to diverse abiotic stresses can discover master miRNAs that control multiple pathways and identify miRNA-specific abiotic stress pathways. Moreover, it will be relevant to study miRNAs involved in other biological pathways such as flowering, plant structure, and pathogen response, which are also critical in the productivity of tef. Hence, using miRNAs and their target genes, the tolerance of tef plants to environmental stresses will improve and thereby productivity will increase.

### Proteomics in tef

Seed storage proteins (SSPs) play a vital role in human nutrition. A study in two cultivars of tef shows that the main SSPs are prolamins, which are referred to as eragrostins [64]. The same study showed that tef has  $\gamma$ - and  $\delta$ -prolamins but no  $\beta$ -prolamins. Another study showed that brown tef contains higher amounts of essential amino acids than white tef, while both tef types

are high in the amino acid lysine [65]. The white and brown tef types also had significant differences in phenolic compounds after industrial processing. Specifically, white tef mostly contained apigenin-derived flavones, while brown tef contained luteolin-derived flavones [66,67].

The first proteomic analysis in *tef* was obtained from a brown-seeded variety and focused on the response under dehydration conditions. Proteomic analysis from the leaf samples using isobaric tag for relative and absolute quantification (iTRAQ) mass spectrometry detected 5727 proteins, of which 211 were differentially expressed under dehydration stress [68]. Further, 57 proteins were produced by alternative splicing and thus can be candidates for drought response according to previous references. **Gene Ontology (GO) term** analysis showed that genes involved in biotic and abiotic stress responses, signaling, transport, cellular homeostasis, and pentose metabolic processes were enriched in upregulated proteins. Whereas terms linked to reactive oxygen species (ROS)-producing processes under water-deficit conditions, such as photosynthesis, associated light-harvesting reactions, cell wall catabolism, manganese transport and homeostasis, the synthesis of sugars, and cell wall modification, were enriched in downregulated proteins [68].

### Metabolomics in tef

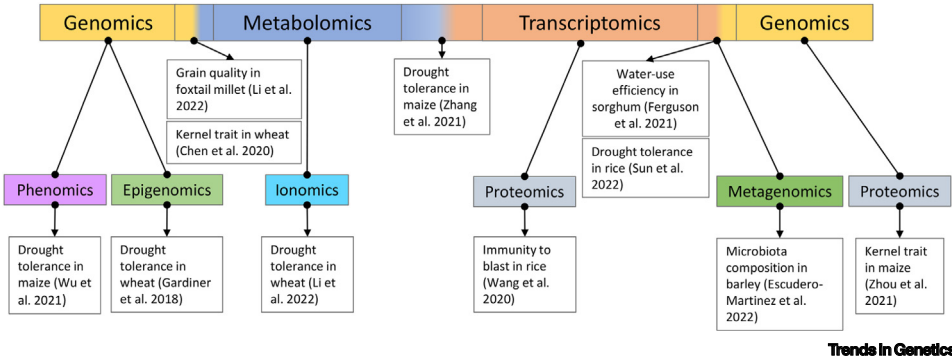
Metabolomics refers to the identification and quantification of metabolites, which are small molecules produced during metabolism. A recent study grouped tef genotypes into four metabolomic classes based on the accumulation of flavones and flavonols [69]. While white-seeded tef showed variations related to sucrose and essential vitamins, nicotinamides (vitamin B3), riboflavin (vitamin B2), and folate (vitamin B9), brown-seeded tef showed variations in metabolism related to amino acid and sugars [69].

Another study by the same team investigated the changes in metabolites in 11 tef accessions exposed to drought conditions. The results showed that flavonoid-associated metabolites and tricarboxylic acid (TCA) intermediates were lower in the drought group, whereas most stress-responsive amino acids and sugars were elevated [70]. However, high accumulation of flavonoids, amino acids (serine and glycine), sugars (ribose, myoinositol), and fatty acids were found in an *Enatite* accession, indicating its elevated tolerance to drought. A similar study suggested that the induction of the three amino acids is related to nitrate assimilation [71]. While fatty acids are associated with the biosynthesis of jasmonate, a drought signal hormone [72], CaMIPS2 enhances tolerance to salinity and drought [73].

A recent study using NaCl treatment on germinating seeds of 19 tef accessions revealed significant metabolic differences between shoots and roots, with roots of salt-tolerant lines accumulating flavonoid derivatives, sugars, and cell wall-associated metabolites [74]. Hence, flavonoid accumulation in roots can be used as a criterion in breeding tef for salinity tolerance.

### Toward multiomics in tef improvement

**Multiomics** refers to a holistic approach of integrating datasets of different omics studies to improve traits in plants [75,76]. Omics that can be used for this purpose include genomics, transcriptomics, proteomics, metabolomics, **ionomics**, **metagenomics**, and **phenomics**. While ionomics investigates the complete mineral nutrient and trace elemental composition of a plant, metagenomics studies communities of microbial organisms directly in their natural environments. Phenomics is the study of observable traits in the organism. Multiomics has been successfully implemented in various crops and traits (Figure 3). In these cases, the combined use of omics enabled researchers to develop drought-tolerant sorghum [77], wheat [78,79], rice [80], and maize [81,82]. Similarly, multiomics was implemented to develop improved grain quality in maize [83] and wheat [84]. Disease resistance and other important traits were incorporated in foxtail millet [85], rice



**Figure 3. Examples of multiomics in improving various traits in cereal crops.** Genomics and phenomics in maize (Wu *et al.* 2021; [81]); genomics and epigenomics in wheat (Gardiner *et al.* 2018; [78]); genomics and metabolomics in wheat (Chen *et al.* 2020; [84]) and foxtail millet (Li *et al.* 2022; [85]); metabolomics and ionomics in wheat (Li *et al.* 2022; [79]); metabolomics and transcriptomics in maize (Zhang *et al.* 2021; [82]); transcriptomics and proteomics in rice (Wang *et al.* 2020; [86]); transcriptomics and genomics in sorghum (Ferguson *et al.* 2021; [77]) and rice (Sun *et al.* 2022; [80]); transcriptomics, genomics, and metagenomics in barley (Escudero-Martinez *et al.* 2022; [87]); and genomics and proteomics in maize (Zhou *et al.* 2021; [83]).

[86], and barley [87]. In addition, omics contributes to the development of resilient crops against climate change [88].

The multiomics approach can also be implemented in tef improvement. Instead of working on a single omics group, a tef plant with trait(s) of interest can be thoroughly researched by the application of multiomics. This can be done by collaboration with researchers in different omics groups.

### The challenge of applying omics in tef improvement

Different types of omics have potential in tef improvement. However, it is not easy to apply omics in developing countries due to constraints related to the high cost of equipment, lack of supplies, and maintenance. In addition, the analysis and interpretation of omics data require proper tools and experts in bioinformatics.

Due to the earlier-mentioned constraints in Ethiopian research laboratories, studies on tef omics have been conducted in laboratories outside the country; for instance, TILLING in the University of Bern in Switzerland and University of Georgia in the USA; metabolomics in Aberystwyth University in the UK; proteomics in the University of Cape Town in South Africa; transcriptomics in the University of Bern; and two genome sequencings by the University of Bern and Michigan University in the USA. The only successful tef genome editing was performed by Dan Forth Center in the USA.

### Concluding remarks

Tef can be considered a model crop due to its resilience to challenging environmental conditions, particularly excess or scarce soil moisture. Due to adaptation to adverse environmental conditions, tef is popular with growers and is considered to be the crop of choice to cope with climate stress. In addition, due to its high nutritional content and health-related benefits, tef is famously known as a superfood.

Tef genome and transcriptome sequencing have been used to develop diverse genetic markers, which were then used in diversity studies. So far, the whole genome has been sequenced for only two tef genotypes. To identify genetic diversity among tef populations and exploit this diversity in

### Outstanding questions

Tef possesses a number of desirable traits, including those related to resilience to environmental stresses. However, its potential for improvement remains untapped due to limited availability of genomic and molecular tools that support modern breeding. It is essential to apply contemporary omics to investigate how tef is resilient to contrasting moisture regimes.

More than 6000 tef accessions collected from diverse agroecologies are available in the gene bank of the EBI. However, only a fraction of these germplasms have been properly characterized. Pangenome or resequencing of tef genotypes needs to be done to capture diversities in tef germplasm.

New breeding technologies (NBTs) including genome editing have high potential in tef improvement as an avenue to address its main limitations, particularly in increasing the standing ability of the stalk and enhancing the nutritive value. However, in some countries genome-edited products are treated similarly to those from transgenics that confront lengthy and expensive biosafety regulations. Hence, scientists need to convince policy makers that genome-edited products are different from transgenics so that tef lines with improved traits can easily be incorporated to the breeding program.

Tef is an African orphan crop, but a large part of the omics research conducted on it has been outside the continent. Recently, a growing number of African scientists are trained in omics. Hence, the effectiveness and efficiency of the different omics and multiomics tools need to be exploited in enhancing the productivity and nutritive value of tef.

Although tef is rich in calcium, protein, and iron, supplementation with zinc (Zn) is required as tef is low in Zn. Studies need to explore the germplasm diversity in Zn nutrition and the potential of using biofortification to enhance the nutrition of tef.

future crop improvement, resequencing of a large number of tef germplasms from representative growing sites and with distinct phenotypes is needed (see [Outstanding questions](#)). The use of many genomes in genomic diversity studies is also known as a **pangenome** approach [89].

Genomics also has a huge potential in tackling major constraints related to tef. The major constraint of tef production is the plant's susceptibility to lodging or the permanent displacement of the stalk from the upright position. Successes have already been noted in the development of semidwarf and lodging-tolerant tef lines from EMS-mutagenized populations [24] and using genome editing, particularly CRISPR/Cas9 [22]. Using molecular markers, substantial diversity has been found for several useful traits. Currently, these diversities have been exploited in tef breeding.

Comparative genomics can also play a crucial role, as tef researchers can exploit information derived from other plants that have already benefitted from advanced genomics. However, the use of this approach requires validation of the biological functions of candidate genes in tef.

Plant proteins and metabolites, which play a vital role in maintaining cellular homeostasis and regulating physiological changes to better adapt to prevailing environmental stresses [90], can also contribute to the advancement of tef research. Studies made so far with limited numbers of tef genotypes and only under drought conditions [68–70,74,91] need to be investigated using representative tef germplasms and under diverse environmental conditions. In this case, multiomics-based tef improvement will significantly improve productivity and resilience against environmental challenges as well as enhance nutritional value.

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### Declaration of interests

No interests are declared.

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