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Tef: A tiny grain with enormous potential

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Abstract

Tef is a highly nutritious gluten-free Ethiopian cereal with food-feed potential. Its productivity however is affected by lodging, weed infestation, terminal drought, small seed size, and shattering. Following the recent availability of tef genome sequences, we highlight the need to harness the benefits that this underutilized crop offers to improve food security.

Breaking the Green Revolution barriers: Adopting forgotten crops for the future

An increasing global population demands more nutritious food and also feed for improved livestock production. Plant-derived foods are the major source of nutrition, and humans mainly depend on cereal grains (i.e. wheat [*Triticum aestivum*], rice [*Oryza sativa*] and maize [*Zea mays*]) for their major dietary requirements [1]. Recently, a wide range of nutrient-rich crops including oats (*Avena sativa*), quinoa (*Chenopodium quinoa*) and millets (foxtail [*Setaria italica*], pearl [*Pennisetum glaucum*]) have become popular as healthy foods [2]. However, other "**orphan crops"** (see Glossary) have potential to contribute to human and animal diets. One such Ethiopian orphan cereal gaining attention due to its health and nutritional benefits is 'Tef' (*Eragrostis tef)*.

Native to Ethiopia, tef is a nutrient-rich cereal with slow-digestible starch, high amino acid, protein, and fibre content [3]. The gluten-free nature and low **glycemic index** of tef grains serves as an alternative cereal to individuals suffering from gluten intolerance and diabetes [4]. Normalising for weight, tef grains contain more minerals (calcium, magnesium, iron, phosphorous and potassium) than in maize, sorghum (*Sorghum bicolor*), wheat and barley (*Hordeum vulgare*), and it is proposed to provide the ideal dietary source to combat malnutrition among children and women in Ethiopia and Eritrea [5, 6]. Tef could be a food crop in other developing and developed countries as part of a healthy diet, but this opportunity has yet to be extensively exploited. The composition and **palatability** of tef straw also makes it an excellent source of animal feed and it is also traditionally used for plastering the walls of houses [3]. Here we highlight the current state of knowledge of tef and also indicate the challenges ahead before it can be fully exploited as a sustainable crop globally.

Tef: Indigenous crop of Ethiopia

Tef is an ancient cereal, domesticated by Ethiopian farmers approximately 6000 years ago. There, it is a major staple cereal [5] and is valued by Ethiopian smallholder farmers for both its grain and straw [7]. Tef is a C4 warm-season annual crop from the Chloridoideae subfamily of grasses and is the only *Eragrostis* species cultivated for human consumption. It is a resilient crop, well adapted to a wide range of environmental conditions growing in temperatures ranging between 10 °C - 27 °C. However, it performs best when grown between altitudes of 1700 - 2500 metres above sea level with annual and growing season rainfalls of 750 - 850 mm and 450 - 550 mm [5].

The word tef comes from the Amharic word "teffa" which means "lost" owing to its tiny seed size with an average length of 1 mm. The grains are oval shaped and vary in colour from ivory white to dark brown. Unlike other cereals, its grains are consumed as a whole grain (including bran and germ) which contributes to its high fibre and protein content. White tef has high consumer preference and fetches the highest price, whereas coloured (red/brown) tef is sold at a low price and consumed locally [6]. However, currently coloured tef is gaining popularity among the health-conscious consumers for its high polyphenols and tannins content which can be a source of antioxidants with anti-diabetic and anti-cancer properties. See **Table 1** for nutrient composition of cooked tef with other major and minor cereals.

Tef has a short life cycle and it can be harvested multiple times in a year. This generates a large quantity of straw which can be used as forage and for industrial purpose. Tef straw is mainly used as animal feed in Ethiopia and is cultivated as forage crop in South Africa and in the Republic of Korea [8]. Tef leaves are long and slender, due to its highly digestible carbohydrates and low-cost, tef hay is used as a replacement for alfalfa (*Medicago sativa*) to feed horses, sheep and dairy cows in the United States [9]. Tef straw is also used as a raw material to produce biomethane and biogas that can be used as biofuels to reduce greenhouse gases emission.

Bottlenecks in Tef: Lack of fundamental and advanced research

Despite the benefits of tef, considerable impediments to its productivity remain. Most obvious is the very small grain size, which is a problem and limitation during planting and harvesting. Other production constraints are also associated with low tef yields and commercial productivity, including **seed shattering**, **lodging,** weed infestation and lack of stress tolerant genotypes **(Figure 1)**. This gap can now be addressed through the application of advanced molecular tools for the evaluation and selection of promising tef varieties from the existing 6797 germplasm collections available globally. The Ethiopian Biodiversity Institute alone holds about 6000 accessions collected from different regions in Ethiopia or donations from individuals, local and international institutions [5]. Tef research in Ethiopia has so far released over 50 improved varieties adapted to extreme environmental conditions, typical to Ethiopia such as high rainfall, low rainfall and highland waterlogged areas. However, the diversity in tef germplasm remains to be exploited, with systematic traits assessments very much in their infancy. Furthermore, there has been a paucity of fundamental research on tef due to lack of funding. For example, there have been no in-depth assessments of germination and senescence within tef populations. This could be addressed by adopting multi-disciplinary systems biology approaches for selecting candidate genes or regulatory pathways that control positive (i.e. nutrition-high iron content, adaptability-tolerance to waterlogging/drought) and negative traits (i.e. grain size, lodging) in tef. Two such candidates associated with lodging tolerance and plant height are semi-dwarf (*SD1*) and reduced height *(RHT*) genes from rice and wheat [10]. The homologs of these genes were sequenced from 31 tef accessions and could be used to develop semi-dwarf tef varieties using gene-editing approaches [11]. Another factor that limits mineral bioavailability is **phytic acid** [4]. This is particularly relevant when considering the consumption of tef sprouts as micro greens, where phytase activities could reduce the levels of phytic acid. However, further studies are required to correlate the role of phytic acid and mineral absorption.

The foundation for molecular and genetic studies has been laid with the completion of several genome sequences. Tef is an allotetraploid (2n=4x=40) with a genome size of ~ 622 Mbp and a monoploid genome size of 300 Mbp. A draft tef genome was published in 2014 (for Tsedey, an improved cultivar) and a chromosome scale assembly of the tef cultivar ‘Dabbi’ [9] was reported in 2020. Comparison of 32 complete **plastomes** of tef accessions revealed a low level of sequence variability and the variable sites could be used as polymorphic markers for breeding and population genetics [12]. The relatively small monoploid genome size should facilitate the development of elite tef lines based on exploiting its genetic diversity. This requires sequencing more genotypes including its wild relatives, which could provide genomic sources of key allelic variants that can be exploited in breeding programmes linked to **genome-wide association studies (GWAS)**. Omics studies in tef are also being reported now. A recent study compared drought tolerant Tsedey and Alba (susceptible cultivar) to suggest the role of **microRNA**’s and transcription factors in regulating drought responses [13], but these studies need to be expanded. Investigating stress-associated mechanisms that contribute to ‘yield’ using multi-omics systems (genome, transcriptome, metabolome) would provide powerful insights into tef genotypes. This will require a large phenotyping effort combined with the ‘omics’ tools to fully characterize tef diversity. Crop **phenomics** approaches are now well placed to aid such efforts [14] and there are emerging precedents from rice on how wild varieties can be exploited [15]. Beyond phenomics informed GWAS, it is not possible to link metabolomic features to genomic variation. Such metabolic GWAS (mGWAS) approaches could be used to target key nutritional or livestock feed traits in genetic tef variants that could be used for breeding programmes.

Concluding remarks

Tef rich in nutritional and agronomic traits has considerable potential as a gluten-free crop. There are key trait impediments that are preventing its full exploitation in Ethiopia and other countries. However, the foundations for an accelerated tef breeding programme have been established, with an expansive germplasm collection, several genome sequences and the first characterizations of key traits such as drought tolerance. With global climate change, posing even more challenges in ensuring food security, efforts for combined fundamental and advanced research on tef should be increased. These efforts will contribute to make orphan crops like tef available as a sustainable crop for healthier humans and livestock, and will also aid in boosting the economy of developing countries like Ethiopia.

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Declaration of interests

No interests are declared.

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Table 1. Nutrient composition in cooked tef grains compared with other major and minor cereals.

1 cup of cooked grains weight in grams (g) [a]	Tef 252 g	Millet 174 g	Wild rice 164 g	Oat bran 219 g	White rice 158 g	Corn pasta 140 g	Whole Wheat pasta, spaghetti 151 g
Calories [b]	**254.62**	207.06	165.64	87.6	203.82	176.4	224.99
Net Carbs	42.95	38.89	32.0	19.35	43.62	32.37	39.51
Fibre	7.1	2.3	3.0	5.7	0.6	6.7	5.9
Protein	9.75	6.11	6.54	7.03	4.22	3.68	9.04
Fat	1.64	1.74	0.56	1.88	0.44	1.02	2.58
Essential Amino acids							
Isoleucine	0.37	0.26	0.27	0.25	-	0.13	0.35
Leucine	0.78	0.78	0.45	0.52	-	0.45	0.62
Lysine	0.28	0.12	0.28	0.28	-	0.10	0.20
Methionine + Cysteine	0.31	0.12	0.20	0.13	-	0.08	0.15
Histidine	0.22	0.13	0.17	0.15	-	0.11	0.21
Phenylalanine + Tyrosine	0.85	0.51	0.60	0.59	-	0.33	0.69
Threonine	0.38	0.20	0.21	0.19	-	0.14	0.24
Tryptophan	0.10	0.07	0.08	0.12	-	0.03	0.12
Valine	0.50	0.32	0.38	0.36	-	0.19	0.39
Vitamins and Minerals [c]							
Thiamine	0.46	0.18	0.09	0.35	0.26	0.07	0.24
Vitamin B6	0.24	0.19	0.22	0.06	0.14	0.08	0.14
Calcium	123.48	5.22	4.92	21.90	15.80	1.40	19.63
Copper	0.57	0.28	0.20	0.14	0.11	0.09	0.34
Iron	5.17	1.10	0.98	1.93	1.88	0.35	2.60
Magnesium	126	76.56	52.48	87.60	18.96	50.40	81.54
Manganese	7.21	0.47	0.46	2.11	-	0.21	2.00
Phosphorus	302.40	174	134.48	260.61	67.94	106.40	191.77
Potassium	269.64	107.88	165.64	201.48	55.30	43.40	144.96
Zinc	2.80	1.58	2.20	1.16	0.77	0.88	2.02

[a]Data extracted from https://www.nutritionvalue.org/ based on USDA standard reference. The values of carbohydrates, fibre, net carbs, fat, protein, essential amino acids are in grams (g), and all other vitamins and minerals represented as milligrams (mg) values.

[b]Compared to gluten free grains especially to oats and millets, tef has high calorific value rich in carbohydrates, proteins and fibre.

[c]Tef grains possess high levels of vitamins (thiamine, riboflavin) and minerals like calcium, iron, magnesium, manganese, phosphorus, potassium and zinc

Figure legend

Figure 1. shows the limitations of tef and a road map to improve it as a sustainable food and feed crop. Tef is a low yielding crop due to major challenges such as small seed size, seed shattering, low tolerance to lodging, weed and drought. To overcome these constraints, and accelerate the generation of improved tef varieties, basic (developmental and physiological) and advanced (genomics, transcriptomics, and metabolomics) research is needed to explore the genetic diversity and agronomic traits of tef. The figure was created using Biorender (https://biorender.com/).

Glossary

Glycaemic index: describes how quickly a given food will affect blood glucose levels after its consumption.

Germplasm: includes resources such as seeds that can be used for breeding and research purposes

GWAS: Genome wide association studies is an approach that test for variations across the genomes within a population to find phenotype-genotype associations.

Lodging: is the displacement of stem or root of plants from their vertical placement.

m-GWAS: is an integrated study that link metabolite to genetic variations and correlate to biochemical mechanism or phenotype

Micro RNA: are class of small non-coding RNA molecules that are involved in regulating the gene expression

Orphan crops: these are a group of crops which are grown, eaten at a given geographical locale but has received less attention in terms of varietal improvement and research.

Palatability: is referred as quality o f food or forage characteristics like texture, smell and taste.

Phenomics: is the acquisition of high-dimensional computerised image data to understand the natural variation within a population

Plastome: is the genome of a plastid (chloroplast DNA), which is a type of organelle found in plants and range from 115 – 165 kb in size.

Phytic acid: is a natural substance found in plant-based foods which affect the absorption of minerals like iron and calcium which can cause mineral deficiencies.

Seed shattering: is the shedding of mature seeds when they are ripe.