

Technology generation to dissemination: lessons learned from the tef improvement project

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Abstract Indigenous crops also known as orphan crops are key contributors to food security, which is becoming increasingly vulnerable with the current trend of population growth and climate change. They have the major advantage that they fit well into the general socio-economic and ecological context of

developing world agriculture. However, most indigenous crops did not benefit from the Green Revolution, which dramatically increased the yield of major crops such as wheat and rice. Here, we describe the Tef Improvement Project, which employs both conventional- and molecular-breeding techniques to improve tef—an orphan crop important to the food security in the Horn of Africa, a region of the world with recurring devastating famines. We have established an efficient pipeline to bring improved tef lines from the laboratory to the farmers of Ethiopia. Of critical importance to the long-term success of this project is

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the cooperation among participants in Ethiopia and Switzerland, including donors, policy makers, research institutions, and farmers. Together, European and African scientists have developed a pipeline using breeding and genomic tools to improve the orphan crop tef and bring new cultivars to the farmers in Ethiopia. We highlight a new variety, *Tesfa*, developed in this pipeline and possessing a novel and desirable combination of traits. *Tesfa*'s recent approval for release illustrates the success of the project and marks a milestone as it is the first variety (of many in the pipeline) to be released.

Keywords *Eragrostis tef* · Marker-assisted breeding · Orphan crops · Tef · TILLING · Farmer-participatory research

Introduction

Orphan crops, also known as under-researched crops, play a particularly important role in food security, nutrition, and income generation for resource-poor farmers and consumers in developing countries, especially in Africa. These crops perform better than the major crops of the world under the extreme soil and

climatic conditions prevalent in the developing world. Orphan crops are also compatible with the agro-ecology and socio-economic conditions of the regions in which they originate and are grown.

African orphan crops have a number of advantages in terms of adapting to adverse climatic and soil conditions. Among cereals, finger millet (*Eleusine coracana*), fonio (*Digitaria exilis* and *D. iburua*), and African rice (*Oryza glaberrima*) are well adapted to drought-prone areas where moisture is low and are able to sustain normal growth until maturity (NAP 1996; Williams and Haq 2000). Due to their early-maturing properties, most of these crops are the main source of food during critical food shortage periods, particularly the time just before most crops are ready for harvest. Among widely cultivated legumes in Africa, cowpea (*Vigna unguiculata*) is tolerant to drought and heat and grows quickly (Valenzuela and Smith 2002) while grass pea (*Lathyrus sativus*) is considered to be an insurance crop since it produces reliable yields when all other crops fail due to extreme drought. Similarly, several indigenous root crops cultivated in Africa are also resilient to diverse environmental stresses.

In addition to their significant economic importance, African crops provide a nutritive and healthy diet. For instance, the seeds of fonio are nutritious and

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notably rich in methionine and cysteine, two amino acids essential for human health but deficient in major cereals such as wheat, rice and maize (IPGRI 2004). Bambara groundnut (*Vigna subterranean*) is unique since it contains adequate quantities of protein (19%), carbohydrate (63%), and fat (6.5%) (NAP 2006). Finger millet is a popular food among diabetic patients because of its slow digestion and low glycemic index (Chandrashekar 2010). Finger- and pearl-millet have anti-cell-proliferative properties, and might have a potential in the prevention of cancer initiation (Chandrasekara and Shahidi 2011). The orange pulp type banana has high carotenoid and iron contents. Its incorporation into the diets in East Africa has been shown to reduce Iron Deficiency Anemia (IDA) by over 50% and effectively combat Vitamin A Deficiency (VAD), both of which affect a large percentage of the population (Fungo 2009).

Although African indigenous crops perform better than major crops of the world under extreme environmental conditions and fit to the socio-economic conditions of the areas in which they are grown, they also encounter a number of constraints. The major bottleneck is related to the dearth of genetic investigations made on these crops. Almost all orphan crops are being studied by poorly funded researchers based in resource-limited developing nations. The majority of these researchers have little chance to establish partnerships with the scientific community especially with those in the developed world. Modern improvement techniques are rarely employed in orphan crops, while breeding is mostly dependent on the conventional improvement techniques such as selection and hybridization. Only limited numbers of breeders implement modern techniques such as marker-assisted breeding. Genomic information such as whole-genome sequencing is not yet available for most orphan crops.

The famous Green Revolution, which contributed to a significant boost in crop production and productivity in Asia, did not occur in Africa, mainly due to the exclusion of major African staple food crops as a primary focus of improvement (Ejeta 2010; Godfray et al. 2010). In terms of area of cultivation, the top three crops in Africa are maize, sorghum and millet, while in Asia they are rice, wheat and maize in descending order (FAOSTAT 2016). Investment in research and development especially in human resources and infrastructure are also low in Africa.

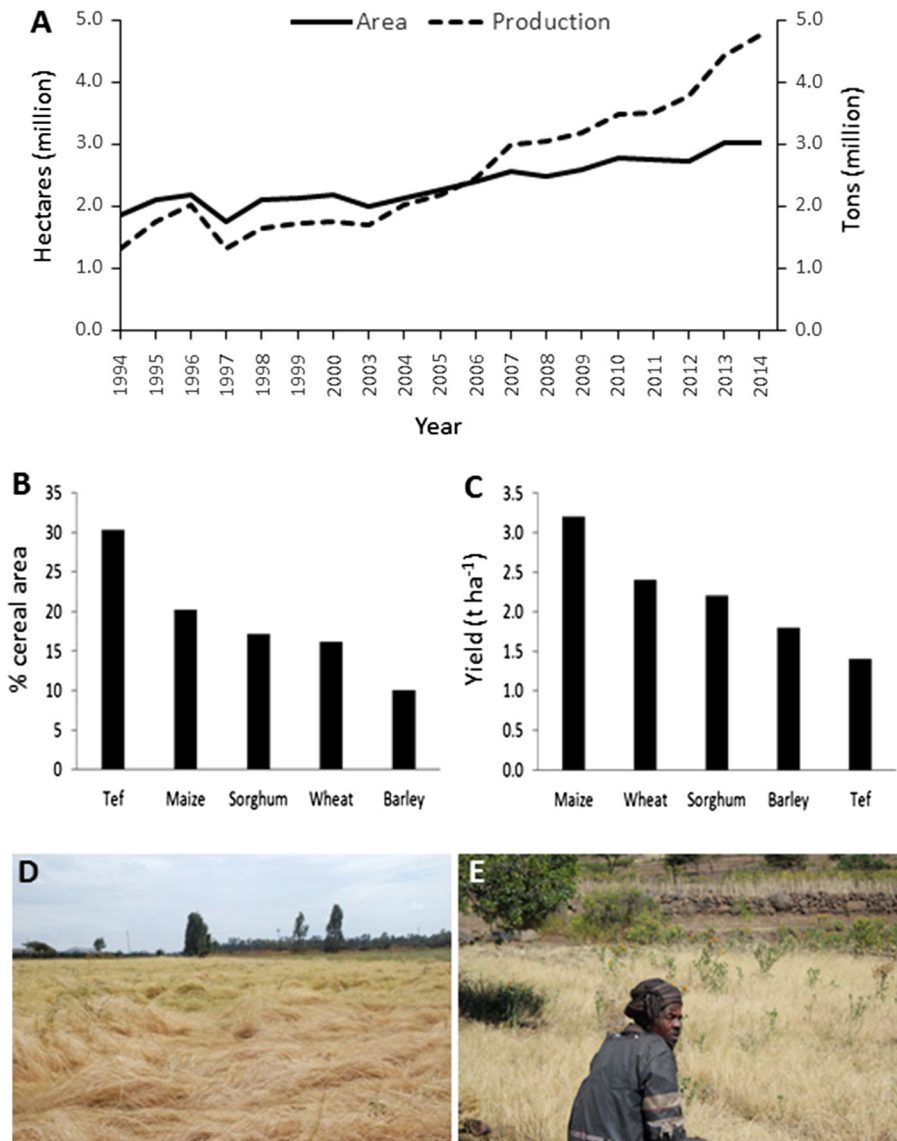
Although African countries agreed to allocate at least 10% of their national budgetary resources to agriculture and rural development policy implementation, only few countries have reached the target (AU 2005).

This review describes the structure and progress of an initiative to improve tef, one of the most important indigenous and understudied crops in the Horn of Africa. The Tef Improvement Project (TIP) brings together scientists, charitable organizations, business leaders and development workers from different countries and institutions for the common goal of providing new improved tef cultivars to farmers in a timely manner. In this review paper, we show that by working together with different stakeholders in a value-chain approach from basic research at the University of Bern to variety development and dissemination in Ethiopia, we can contribute to raising productivity of under-researched crops like tef. The system which we have established can be applied to other systems and crops.

Tef: a crop of choice for scientific research

Tef [*Eragrostis tef* (Zucc.) Trotter] is the most important cereal crop in the Horn of Africa, especially in Ethiopia, where the last two decades have seen tremendous increases in both the total area devoted to the crop and its production (Fig. 1a). It is annually cultivated on over three million hectares of land, which is equivalent to 30% of the total area allocated to cereals (CSA 2015) (Fig. 1b). Tef is preferred both by farmers and consumers. Farmers prefer cultivating tef to other cereals since it is more resilient to environmental stresses such as poor soil drainage during the rainy season and moisture scarcity. In addition, as a cash crop, both the grain and straw of tef fetch higher prices than the respective products from other cereals. Consumers prefer tef not only because it makes good quality “*injera*”, a pancake-like soft bread, but also because it is nutritious due to its high protein and mineral content (Bultosa et al. 2002; Abebe et al. 2007). Furthermore, the absence of gluten (Spaenij-Dekking et al. 2005) makes tef an alternative food for people suffering from celiac disease. Due to this life-style feature of the crop, it has been heralded as a ‘superfood’ or ‘super grain’ (Jeffrey 2015; Provost and Jobson 2014). Overall, tef plays a vital role in food

Fig. 1 Importance and constraints of tef cultivation in Ethiopia. **a** both the total area and the total grain production of tef have significantly increased in the last two decades; **b** tef occupies the highest proportion of cereal area; **c** the productivity per unit area was the lowest for tef compared to other cereals. Lodging or susceptibility of the plant to fall over on the ground **d** and drought **e** are major constraints to increasing tef productivity. **b, c** are from 2014. **a–c** adapted from CSA reports



security, nutrition, and income generation to small-holder farmers in Ethiopia.

Despite its versatility in adapting to extreme environmental conditions, the productivity of tef is low in Ethiopia at 1.5 tons ha^{-1} as compared to 3.2 tons ha^{-1} for maize (CSA 2015) (Fig. 1c). The major yield-limiting factors to tef production are the lack of cultivars tolerant to lodging and to prolonged drought (Assefa et al. 2011) (Fig. 1d, e), as well as tef's small seed size. The low productivity is also related to the widespread use of landraces and cultivars lacking desirable agronomic traits.

Lodging (permanent displacement of the stem from the upright position) is the major production constraint in tef. Tef possesses tall and weak stems that easily succumb to lodging caused by wind or rain. In addition, lodging hinders the use of high input husbandry since increasing the amounts of nitrogen fertilizer to boost the yield also exacerbates the lodging problem. Consequently, both the yield and the quality of the grain and the straw are severely reduced. Lodged plants are difficult to harvest manually and make mechanical harvesting impossible.

Genomic information such as whole-genome sequencing has not been available until recently for

most orphan crops including tef, a self-pollinated, allotetraploid plant for which the true diploid ancestors are not yet known. Since the genomic sequences of grass family members are somewhat conserved, interesting genes from orphan crops can sometimes be identified from orthologous genes in related crops. The phylogenetic tree of the grass family in Fig. 2 shows the relationship of tef to other cereals. Finger millet is the most closely related crop to tef as they both belong to the subfamily *Chloridoideae*. Until the completion of the genome sequencing in 2014 (Cannarozzi et al. 2014), the lack of genomic sequences for both finger millet and tef was the major limitation in using genomic tools in tef breeding.

In general, tef is a prime example of an understudied orphan crop since little scientific improvement has been made on the crop compared to the globally major crops such as maize, wheat and rice. This makes tef an attractive target for the implementation of high-tech breeding and genomic strategies. To this end, the Tef Improvement Project was established in July 2006 with the financial support from the Syngenta Foundation for Sustainable Agriculture and the University of Bern in Switzerland, and is hosted at the Institute of Plant Sciences at the University of Bern.

The tef improvement project: goals and strategy

The main aim of the Tef Improvement Project is to boost the productivity of tef by tackling major production constraints through developing cultivars with desirable agronomic and nutritional traits. Hence, the project focuses on problem-oriented or demand-driven research. Specific objectives of the project are:

- (i) To develop tef cultivars with desirable traits using diverse improvement techniques. Priority has been given to lodging and drought, both of which contribute to significant yield loss in tef production.
- (ii) To sequence the genome and transcriptome of tef for use in marker-assisted breeding and high throughput screenings.
- (iii) To study diversity in tef accessions with the aim of identifying natural variation in relevant traits.
- (iv) To disseminate new tef varieties with improved traits to the Ethiopian farming community.
- (v) To contribute to the human capacity building of the Ethiopian Agricultural Research System.

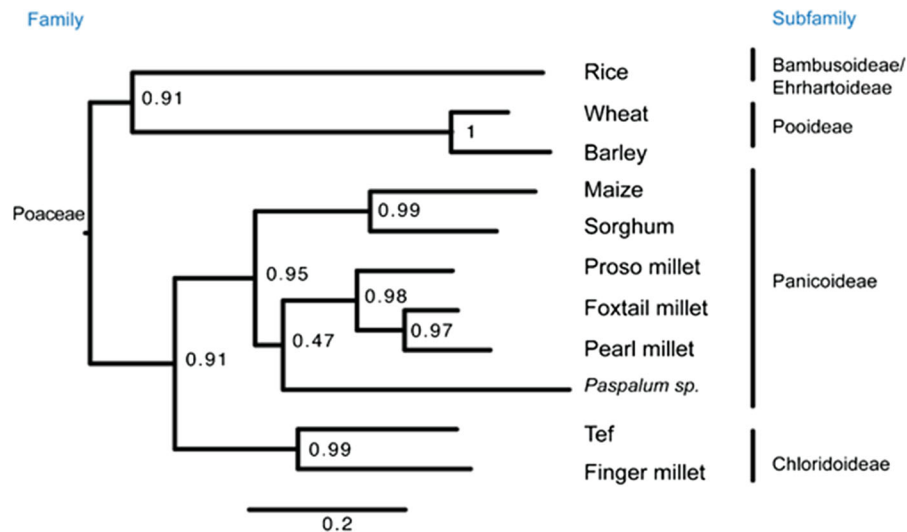


Fig. 2 Phylogenetic tree showing the molecular relationships of tef to other cereals. The tree was constructed using *Waxy* gene from rice (*Oryza sativa*, AF51548), bread wheat (*Triticum aestivum*, HQ338714), barley (*Hordeum vulgare*, GU599884), maize (*Zea mays*, EU041692), sorghum (*Sorghum bicolor*,

EF089839), proso millet (*Panicum miliaceum*, GU199268), foxtail millet (*Setaria italica*, AB089143), pearl millet (*Pennisetum glaucum*, AF488414), *Paspalum simplex* (AF318770), tef (*Eragrostis tef*, AY136935), and finger millet (*Eleusine coracana*, AY508652)

The strategy and pipeline to develop new tef cultivars with valuable traits and to move the new cultivars to the field in Ethiopia are shown in Fig. 3. In the *Technology Generation* process, the project applies modern genetic, molecular and genomic tools to obtain candidate tef lines for the diverse goals of the project. In the Forward Genetics approach, the mutagenized tef population is used for phenotypic screening in order to obtain candidate mutant lines for the traits of interest. Moreover, the same populations are used in Reverse Genetics approaches such as TILLING (Targeting Induced Local Lesions IN Genomes) to screen for mutant lines that harbor DNA lesions in genes of interest. In the *Technology Transfer* phase,

promising tef lines harboring traits of choice are sent to the Ethiopian Institute of Agricultural Research where they are introgressed into high-yielding and widely adapted cultivars and evaluated for several generations at the on-station and on-farm sites across Ethiopia before release to the farming community. The project is also involved in capacity building through short- and long-term trainings, workshop organization and provision of research supplies. In the *Technology Delivery* phase, seeds of newly released varieties are multiplied and disseminated through private and public institutions. The project also focuses on the scaling-up of improved tef technologies where model farmers with know-how in the handling

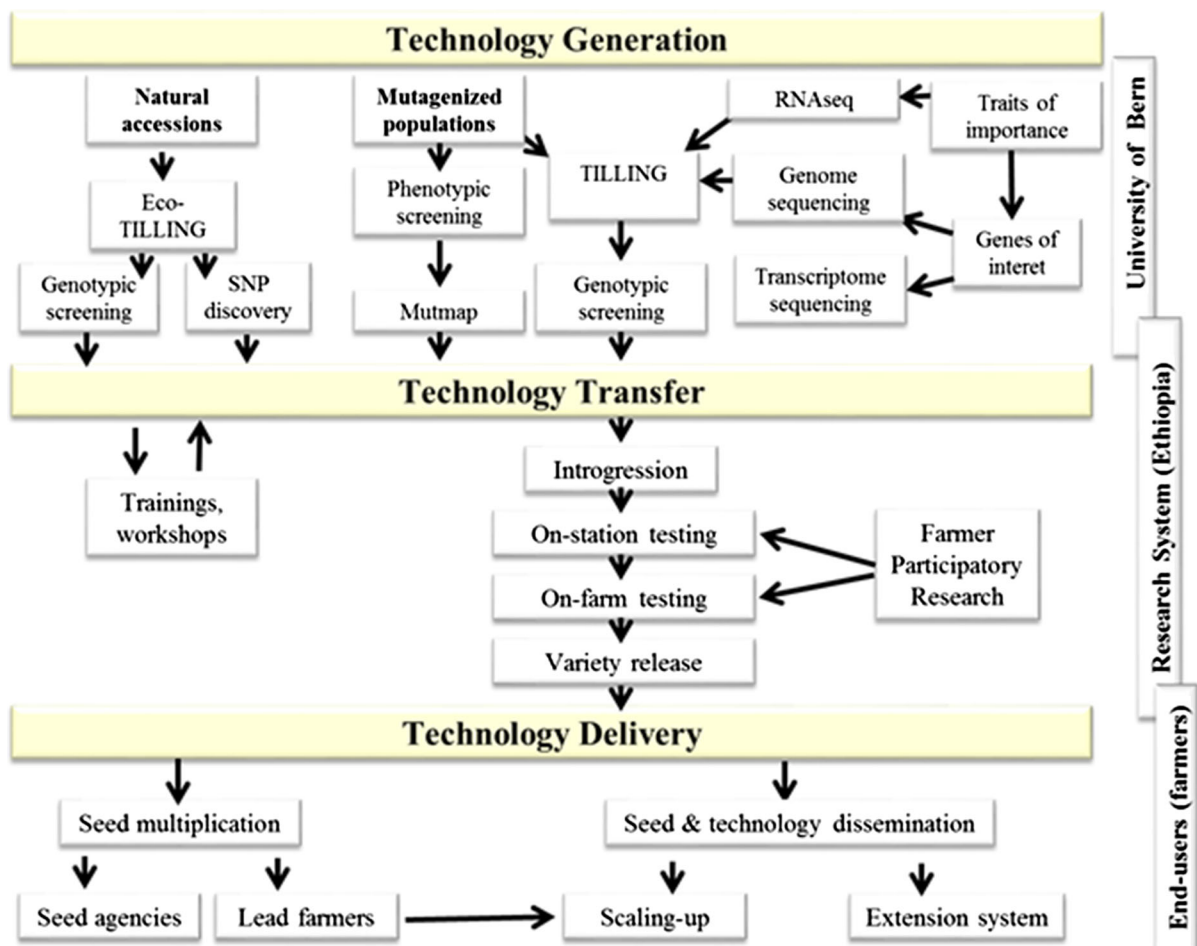


Fig. 3 Strategies and activities of the Tef Improvement Project. The first phase involves technology generation wherein improved cultivars are developed or methods are optimized. In the second phase, promising materials are introgressed to locally-adapted and high-yielding varieties and field-tested in

Ethiopia. Technology delivery refers to both seed multiplication and dissemination as well as dissemination to key partners. The project is also involved in human-resource development through short- and long-term training

of improved seeds play key roles in multiplying and disseminating of seeds and knowledge to fellow farmers.

Tools and resources for technology generation

The Tef Improvement Project implements diverse tools and techniques.

Mutation breeding

Mutation breeding is a process which includes three steps, namely mutation induction, mutation detection and mutation utilization (Tadele et al. 2010). Mutation induction refers to the exposure of seeds or other parts of the plant to chemicals or radiation to induce random nucleotide mutations in the genome. To establish mutagenized populations, seeds of three improved tef varieties, namely *Tsedey* (DZ-Cr-37), *Dukem* (DZ-01-974) and *Kora* (DZ-Cr-438 RIL 133B) were treated with the chemical ethyl methanesulfonate (EMS). The stock of seeds and the DNA from 10,000 mutagenized M₂ populations from these three elite cultivars are available for screening for the trait(s) of interest. Screening of a mutagenized population for candidate lines based on their phenotype is a simple and effective strategy if the desired trait is readily observable. As lodging is one of the main production constraints for tef, screening was first applied to the discovery of semi-dwarf tef lines.

Primary screenings of these mutagenized populations have been made for various agronomic and nutritional traits at several research and higher-learning institutions (Table 1). While the majority of screenings have been done at the host institute at the University of Bern, screening for soil acidity was made at ARARI (Amhara Regional Agricultural Research Institute) in Ethiopia with the collaboration with the University of KwaZulu-Natal in South Africa (Ermias Abate, personal communication), while screening for altered starch content was done at the ETH Zurich (Wuyan Wang, personal communication) (Table 1, Fig. 4).

Tef accessions collected from diverse regions in Ethiopia also exhibited huge diversity for important agronomical and morphological traits (Assefa et al. 2015). A detailed study on 200 accessions collected from diverse agroecologies from major tef-growing

areas showed substantial phenotypic diversity (Habte Jifar, personal communication).

TILLING

TILLING (Targeting Induced Local Lesion IN Genomes) is a high-throughput non-transgenic method that allows for screening single-base mutations in a specific gene over an entire mutagenized population (McCallum et al. 2000). The technique comprises the following steps: (i) mutagenesis, (ii) development of a non-chimeric population, (iii) preparation of a germplasm stock, (iv) DNA extraction and sample pooling, (v) population screening for induced mutations including validation, and (vi) evaluation of candidate mutants for desirable trait(s) (Tadele et al. 2010; Esfeld et al. 2013). Gene targets are found by using genes known to control the agronomic or nutritional traits of interest in other plants and/or from RNA-Seq experiments. Usually, successful candidates arise from knocking out a gene by inducing either a missense mutation or a stop-codon inducing mutation. Tef is an allotetraploid plant and therefore, in order to observe phenotypic effects, recessive mutations need to be induced in each of the homoeologous chromosomes, which then need to be combined by crossing. To find candidates for a semi-dwarf line, TILLING was implemented on 5,000 mutagenized families. Genes implicated in regulating plant height, particularly two genes from the gibberellic acid and brassinosteroid biosynthesis pathways were targeted. Of the 40 lines harboring mutations in either of these two genes, sequencing has thus far revealed 15 mutations with non-synonymous missense or nonsense mutations (Korinna Esfeld, personal communication).

In vitro regeneration

In vitro regeneration has applications in the embryo rescue technique which is important to circumvent the hybridization barrier between tef and wild species as well as in modern improvement techniques including CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) (Miao et al. 2013). We have optimized the technique for tef in which a fully grown plant is developed from immature embryos (Plaza-Wüthrich et al. 2015). Embryo rescue techniques are mostly used in crossing programs involving other

Table 1 Primary screening and status of mutagenized tef populations developed at various Universities for diverse traits

Trait of interest	Background genotype	Institution involved	Screening technique	Status
Semi-dwarfism	Tsedey, Dukem and Kora	University of Bern	TILLING and phenotypic screening	Semi-dwarf and lodging-tolerant variety to be released
Drought tolerance	Tsedey and Dukem	University of Bern	Phenotypic screening	Drought-tolerant variety at the last stage of testing
Soil acidity tolerance	Tsedey	Kwazu-Natal—ARARI	Phenotypic screening	Promising candidate obtained
	Kora	University of Bern	Phenotypic screening	Validation of candidates
Soil salinity tolerance	Kora	University of Bern	Phenotypic screening	Validation of candidates
Herbicide tolerance	Tsedey and Dukem	University of Bern	Phenotypic screening	Validation of candidates
Seed size	Tsedey and Kora	University of Bern	Phenotypic screening	Planned
Starch content	Tsedey	ETH Zurich	TILLING	Validation of candidates

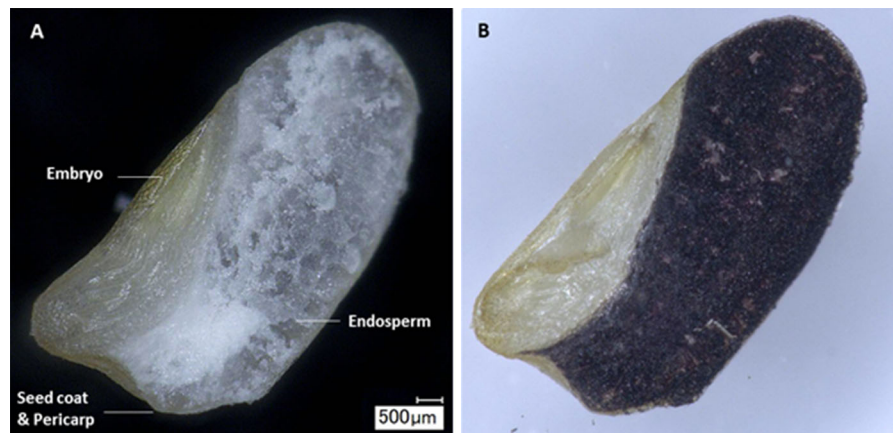


Fig. 4 Starch-rich seed of the tef variety *Tsedey*. **a** A single tef seed cut in half and observed using a VHX-5000 Keyence digital microscope; **b** The same seed after iodine-staining with Lugol's

solution, which stains the starchy endosperm a blue-black color. (Color figure online)

plant species. At the present time, we are introgressing wild *Eragrostis* species with desirable traits to tef which will be followed by rescuing the embryos.

Tef seed pelleting

Seed pelleting involves adding some materials to the seed surface to change their size and/or shape to improve sowing. Seed pelleting is useful in tef husbandry as it facilitates row sowing of the tiny seeds. Figure 5 shows preliminary experiments from increasing the size of tef seeds through pelleting and

the effect of different-sized pellets on the early establishment of tef plants. At present, the pelleting materials are inert and the primary function is in seed handling, however, in the future, pelleting materials can also contain compounds to boost seedling nutrition and to protect the seeds from pests and diseases. The pelleting project is a collaboration among Syngenta AG, Syngenta Foundation for Sustainable Agriculture, University of Bern and the Ethiopian Institute of Agricultural Research. Effort is being made to make the pelleting technology accessible and affordable to smallholder farmers in Ethiopia.

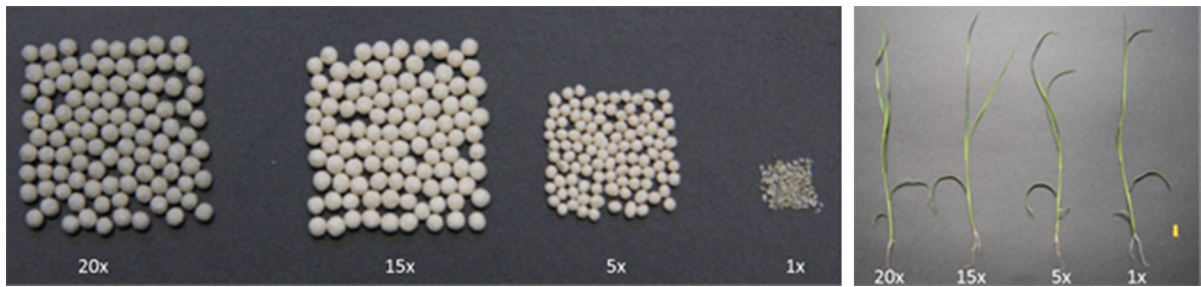


Fig. 5 Seed pelleting is useful in tef husbandry as it facilitates row sowing of the tiny seeds. **a** differing sizes of pelleted seeds by weight ($\times 20$, $\times 15$, $\times 5$) compared to the naked seeds ($\times 1$); **b** seed pelleting has no effect on the growth of tef seedlings. The

Tef Pelleting Project is a collaborative work among Syngenta-Maribo, Syngenta Foundation for Sustainable Agriculture, the University of Bern and the Ethiopian Institute of Agricultural Research

Biomechanics

In order to investigate the lodging-tolerance of diverse tef genotypes, we use a custom-made robot employing Cellular Force Microscopy (CFM) (Routier-Kierzkowska and Smith 2014). With this robot, 3-point bending is used to measure the stiffness of stem sections, while pushing is used to assess root anchorage strength. Information from these techniques is valuable for tef breeders as lodging resistance of the tef plants might be dependent on structural parameters of the plant. We are also collaborating with researchers at the University of Edinburgh, UK, to structurally prime tef plants via mild mechanical stimulation, e.g., stomping (Iida 2014). Such crop management strategies may bring about economical and immediate solutions for enhancing lodging resistance.

Omics

We have implemented a variety of omic techniques which are briefly described below.

Whole genome sequencing

The tef variety *Tsedey* was selected for whole-genome sequencing because it is early maturing and widely adapted. Genome sequencing has the following applications in tef improvement: (i) identification of primer sequences for gene amplification without resorting to other genomes or degenerate primers; (ii) isolation of tef's two homeologous sub-genomes, which is especially useful when designing, genome specific primers for high-throughput techniques such as TILLING; (iii)

designing of genetic markers such as Single Nucleotide Polymorphisms (SNPs) and Simple Sequence Repeats (SSRs) which are used for marker-assisted breeding, for the construction of high density genetic maps and for linkage disequilibrium studies on diverse germplasm and; (iv) understanding the molecular basis of tef's tolerance to many abiotic and biotic stresses. Once obtained from tef, this knowledge can be transferred to other economically important crops.

Transcriptome sequencing

A normalized transcriptome library produced with 454 sequencing was sequenced to estimate the number of genes. In addition, RNA-Seq experiments were conducted using plants grown under control, waterlogging and drought conditions, as well as in developing seeds.

MicroRNA sequencing

The microRNAomes of two tissues (root and leaf) were determined for two tef genotypes subjected to either a two-week drought or normal watering. The two tef genotypes used for the study were a drought-susceptible natural accession called *Alba* and a drought-resistant improved variety called *Tsedey*. For the identification of miRNAs important in the response to drought resistance, responses of the two tef cultivars were compared.

Proteomics

The response of tef to drought was investigated using quantitative proteomics measured with iTRAQ

labeling in combination with mass spectroscopy. These data accompanied classical characterization of the physiological responses of tef to imposed changes in relative water content (RWC), such as electrolyte leakage and chlorophyll fluorescence measurements when tef tissues were dehydrated to 50% RWC as opposed to normally hydrated tissues with 80% RWC.

Technology transfer: introgression and early variety development

Introgression

Once candidate tef lines with desirable traits have been identified, further investigations are carried out in greenhouses and in the field. Confirmed candidate lines are then sent to the Ethiopian Institute of Agricultural Research (EIAR) for crossing, field-testing, and release to the farming community. In order to introduce desirable traits to the tef germplasm of popular and high-yielding lines, lodging-tolerant and drought-tolerant lines have been introgressed to a number of improved tef cultivars grown in Ethiopia. Table 2 shows some of the new candidates, their crosses and their positions in the pipeline. Candidate lines originating from the project have been used in 50% of the crosses in the National Tef Research Program due to their desirable traits such as lodging- and drought- tolerance. These candidate lines have been introgressed to popular improved tef varieties such as *Quncho*, *Kora* and *Magna*.

Early variety development

Different progenies of the crosses are evaluated for several generations at Debre Zeit Agricultural Research Center in Ethiopia. Once initial screenings of the genotypes have been completed, those with enhanced performance are promoted for multi-location evaluation at sites representative of traditional tef growing areas (Table 3). In 2016, 16 experiments, each containing 80 genotypes were tested at 24 representative sites. The sites belong to several research and higher learning institutions and range in altitude from 1400 m asl at Wolenchiti to 2500 m asl at Bichena and Shambu. Hence, they represent the huge diversity in the agroecology of the country. These breeding experiments are designed and dispatched to

collaborating centers via the National Tef Research Program based at the Debre Zeit Agricultural Research Center. Candidate materials with enhanced performance at several locations over several seasons are then promoted to variety verification stage where they are grown on larger plots to be investigated by the National Variety Release Committee. Collaboration with the EIAR has enabled the evaluation of promising lines via on-station and on-farm field-testing through the Farmer Participatory Research program (Belay et al. 2006) where the best performing lines are recommended for a release to the farming communities. Farmers, development agents and other relevant stakeholders are invited to events organized regularly to introduce new tef varieties.

Based on increased demand from both the domestic and global markets for tef grain, the Ethiopian government has recently planned to expand tef cultivation in the western and eastern parts of the country where tef is not usually grown. To identify genotypes best suited for these areas with diverse environmental conditions, for the first time in 2016, 47 tef genotypes from the Tef Improvement Project have been investigated at 10 non-traditional locations out of which five are rain-fed and five irrigated (Table 4).

Achievements of the project

Major successes of the Tef Improvement Project are briefly indicated below.

The first semi-dwarf and lodging-tolerant tef

We have identified and characterized two novel tef mutants (namely *kegne* and *kinde*) both with semi-dwarf stature and lodging-tolerant properties. The mutation in *kegne* is due to a point mutation in the α -*Tubulin 1* gene which co-segregates with the mutant phenotype and serves as a marker in breeding (Jost et al. 2015) (Fig. 6a, Table 2). The *kegne* mutant attains 50 to 70% of the height of the parental line. This modest height reduction is optimal for lodging resistance and grain yield for human consumption as well as maintaining sufficient straw for livestock feed. The putative mutation in *kinde* has been discovered using a modified MutMap technique and is currently being confirmed (Muluneh Tamiru, personal communication). Two lodging-tolerant and semi-dwarf tef

Table 2 Partial view of crosses made to candidate tef lines developed by the Tef Improvement Project

Candidate line	Desirable trait	Crossed to	Used as	Year of crossing	Current status
<i>kegne</i>	Lodging-tolerant	<i>Quncho</i>	M	2010	NVT
		<i>Magna</i>	M	2010	NVT
		<i>Tsedey</i>	M	2010	F ₃ seeds
		<i>Key Murri</i>	M	2011	NVT
		<i>DZ-01-2785</i>	M	2013	F ₇ seeds
<i>kinde</i>	Lodging-tolerant	<i>Quncho</i>	M	2010	NVT
		<i>Key Murri</i>	F	2011	NVT
		<i>Dukem/Quncho</i>	M	2011	F ₃ seeds
		<i>Tsedey</i>	M	2013	F ₄ seeds
		<i>DZ-01-192</i>	F	2013	F ₄ seeds
		<i>DZ-01-192</i>	M	2014	F ₃ seeds
		<i>Rosea</i>	M	2013	F ₄ seeds
		<i>DZ-01-2786</i>	M	2014	F ₃ seeds
		<i>DZ-01-99</i>	F	2014	F ₆ seeds
		<i>DZ-01-2785</i>	F	2914	F ₆ seeds
		<i>Alba</i>	M	2014	F ₆ seeds
		<i>Magna</i>	M	2014	F ₃ seeds
		<i>Boset</i>	M	2014	F ₃ seeds
<i>RIL44</i> (<i>Key Murri</i> x <i>kinde</i>)	Semi-dwarf/lodging tolerance	<i>Dukem</i>	F	2016	F ₁ seeds
		<i>Magna</i>	F	2016	F ₁ seeds
		<i>Magna</i>	M	2016	F ₁ seeds
		<i>DZ-01-99</i>	F	2016	F ₁ seeds
		<i>RIL181</i>	M	2016	F ₁ seeds
<i>RIL302</i> (<i>Key Murri</i> x <i>kinde</i>)	Semi-dwarf/lodging tolerance	<i>Kora</i>	M	2016	F ₁ seeds
		<i>Kora</i>	F	2016	F ₁ seeds
		<i>Magna</i>	F	2016	F ₁ seeds
		<i>DZ-01-99</i>	M	2016	F ₁ seeds
		<i>Dukem</i>	M	2016	F ₁ seeds
<i>dt2</i>	Drought-tolerance	<i>RIL181</i>	M	2016	F ₁ seeds
		<i>Quncho</i>	M	2013	OVT
		<i>Key Murri</i>	F	2013	F ₈
		<i>Kegne</i>	F	2013	F ₃ seeds
		<i>Dtt13</i>	F	2013	OVT
<i>dt13</i>	Drought-tolerance	<i>Magna</i>	F	2013	F ₃ seeds
		<i>Magna</i>	M	2013	F ₃ seeds
		<i>Dtt2</i>	M	2013	OVT
		<i>Magna</i>	F	2013	F ₃ seeds
		<i>Key Murri</i>	F	2013	F ₃ seeds
<i>tdt4-15</i>	Terminal drought tolerance	<i>Bosset</i>	M	2014	F ₃ seeds
		<i>Magna</i>	M	2016	F ₃ seeds
		<i>Kora</i>	M	2016	F ₁ seeds
		<i>Dukem</i>	M	2016	F ₁ seeds

Table 2 continued

Candidate line	Desirable trait	Crossed to	Used as	Year of crossing	Current status
<i>tdt4-19</i>	Terminal drought tolerance	<i>Bosset</i>	M	2016	F ₁ seeds
		<i>Dukem</i>	M	2016	F ₁ seeds
		<i>Kora</i>	M	2016	F ₁ seeds
		<i>RIL44</i>	M	2016	F ₁ seeds

M male, *F* female, *NVT*, national variety trial, *OVT* observation variety trial

cultivars (namely RIL 44 and RIL 302) (Fig. 6b, c) which were obtained by crossing *kinde* with *Kay Murri* (a landrace with a strong stem) have been tested for several seasons at representative locations and will soon be submitted for variety registration.

A new variety with special morphological properties has been evaluated by the National Variety Release Committee and approved for release in March 2017. This release marks a milestone for the Tef Improvement Project as it is the first candidate to obtain formal status as a new variety. The new variety RIL-181 will be named '*Tesfa*', the Amharic word for '*Hope*', based on the suggestion of farmers who evaluated it. The performance of *Tesfa* in terms of grain yield and shoot biomass are shown in Fig. 7. Although the yield and biomass of RIL-181 (*Tesfa*) are comparable to both the popular variety *Quncho* as well as local varieties, the new variety possesses unique and desirable properties including the absence of shattering, a thick and strong culm and compact panicles. These properties, especially the increased culm strength, mean that the new variety can not only be cultivated in rainfed agriculture like other tef varieties, but can also potentially be grown in large-scale irrigated farms with mechanical harvesting. These are the main reasons for the approval of *Tesfa* variety by the National Variety Committee although the height reduction and lodging tolerance of this particular variety were not significantly different from the existing tef varieties (data not shown).

In order to disseminate seeds of the new variety to large numbers of farmers, it has been planned that both public and private sectors will multiply the seed, including lead farmers with experience in producing high quality seed. The Debre Zeit Research Center has a well-established and proven tef technology dissemination system involving multiple sectors and stakeholders.

Drought tolerant tef

We focus on the drought which normally occurs during the early developmental stage of the tef plant when the rain stops for 2–3 weeks soon after crop germination. We selected tef lines that are tolerant to this kind of moisture scarcity while the original tef lines are severely stressed. Screening the mutagenized population enabled us to obtain several candidate tef lines with enhanced drought tolerance (Table 1). Among these, *dtf 2* (drought tolerant tef 2) and *dtf 13* have shown particularly promising performance. The unique morphological difference between these *dtf* lines and the parental line is stomata. The stomata at the adaxial or upper side of the two *dtf* lines are smaller in both size and number compared to the original tef line (Fig. 6d–f). These small-sized stomata in *dtf* lines might contribute towards making the plant more tolerant to drought as less water is lost through transpiration (Sonia Plaza-Wüthrich, personal communication). Both *dtf* lines were introgressed to high-yielding improved cultivars and these introgressions have now reached F₈ and are in Observation Variety Trials (OVT) where they will be tested at multiple sites in drought prone areas (Table 2).

The first tef genome

A draft genome with 47-fold coverage with Illumina HiSeq 2000 and seven-fold coverage with 454-FLX pyrosequencing was generated (Cannarozzi et al. 2014). Assembly and scaffolding resulted in a genome size of 688 Mbp, roughly 95% of the 1C genome size estimated by flow cytometry (Hundera et al. 2000; Ayele et al. 1996). The information from the sequencing is being utilized in identifying and isolating genes responsible for useful agronomic and nutritional traits. A considerable number of genetic markers particularly

Table 3 Traditional tef-growing locations in Ethiopia where germplasms from the Tef Improvement Project have been evaluated under diverse categories in 2016 after crossing to improved tef varieties

Center/site	Institute ^a	Distance and direction from Addis Ababa	Altitude (m a.s.l.)	Climate	Experiments tested ^b
Adadi Mariam	EIAR	55 km S	1900	Sub-humid	16
Adet	ARARI	445 km NW	2240	Moist-cool	16
Akaki	EIAR	10 km S	2300	Cool-wet	1,2,5,6,7,8,9,11,13,14,16
Alem Tena	EIAR	110 km S	1650	Semi-arid	3,4,10,12,15
Ambo	EIAR	115 km W	2185	Temperate	16
Asosa	EIAR	655 km W	1590	Warm to sub-humid	15
Axsum	TARI	955 km N	2100	Semi-arid	15,16
Bichena	ARARI	270 km NW	2500	Cool-wet	16
Chefe Donsa	EIAR	40 km SE	2400	Cool-wet	1,2,5,11,13,14,16
Debre Zeit-light soil	EIAR	45 km S	1800	Temperate	3,14,15,16
Debre Zeit-Black soil	EIAR	45 km S	1800	Temperate	1,2,3,4,5,10,11,12,13,14,15,16
Dhera	EIAR	122 km SE	1680	Semi-arid	6,7,9,15
Hirna	HU	365 km E	1775	Cool-wet	15,16
Holetta	EIAR	35 km W	2390	Cool-wet	16
Ginchi	EIAR	90 km W	2200	Tepid-moist	16
Jimma	EIAR	365 km SW	1760	Sub-humid	16
Mehoni	TARI	635 km N	2400	Arid	15
Melkassa	EIAR	115 km SE	1550	Semi-arid	8,10,15
Minjar	EIAR	110 km SE	1800	Semi-arid to moist	4,10,11,12,13,14,15,16
Shambu	OARI	305 km W	2500	Cool-wet	16
Simada	ARARI	770 km NW	2470	Semi-arid	15
Sirinka	ARARI	510 km N	1850	Semi-arid	15
Wolenchiti	EIAR	120 km SE	1400	Semi-arid	15
Wolkite	WKU	190 km SW	1920	Sub-humid	16

^aInstitutes: *ARARI* Amhara Regional Agricultural Research Institute, *EIAR* Ethiopian Institute Agricultural Research, *HU* Haramaya University, *OARI* Oromia Agricultural Research Institute, *SARI* South Agricultural Research Institute, *TARI* Tigray Agricultural Research Institute, *WKU* Wolkite University

^bExperiments: (1) Observation Variety Trial—Late Set I; (2) Observation Variety Trial—Late Set II; (3) Observation Variety Trial—Early Set; (4) Observation Variety Trial—Semi-dwarf; (5) Observation Variety Trial—Late Set Semi-dwarf; (6) Observation Variety Trial—Drought Tolerance I; (7) Observation Variety Trial—Drought Tolerance II; (8) Observation Variety Trial—Drought Tolerance III; (9) Observation Variety Trial—Drought Tolerance IV (10) Preliminary Variety Trial—Early set I; (11) Preliminary Variety Trial—Late Set IV; (12) Preliminary Variety Trial—semi-Dwarf, Early Set; (13) Preliminary Variety Trial—Late Set III; (14) Preliminary Variety Trial—Semi-dwarf; (15) National Variety Trial—Early set; (16) National Variety Trial—Late Set III-I

SSRs (also known as microsatellites) have been identified (Table 5).

Other key findings in tef in the area of omics are:

Transcriptome

Sequencing of the transcriptome resulted in approximately 38,000 transcripts. Prediction of coding regions resulted in 33,000 genes which were then annotated and for which orthologous genes were found between tef, sorghum, and rice (Fig. 8a) (Cannarozzi et al. 2014).

RNA-Seq

RNA-Seq experiments using waterlogging and drought treatments revealed genes differentially regulated under these two conditions ($p < 0.01$) (Fig. 8b) (Gina Cannarozzi, personal communication). Furthermore, RNA-Seq experiments have been used to analyze the transcriptome of developing seeds to identify genes involved in endosperm starch biosynthesis. These experiments provide targets for TILLING as well as for miRNA analyses.

Table 4 Non-traditional tef-growing locations in Ethiopia where germplasms from the Tef Improvement Project have been evaluated in 2016 after crossing to improved tef varieties

Center/site	Institute ^a	Distance and direction from Addis Ababa	Altitude (m a.s.l.)	Climate
Rain-fed sites				
Abobo	GARI	750 km W	500	Sub-humid
Humera	TARI	975 km NW	600	Warm-moist lowland
Jinka	SARI	580 km SW	1500	Sub-humid lowland
Metema	ARARI	900 km NW	690	Sub-humid
Pawe	EIAR	575 km NW	1000	Sub-humid
Irrigated sites				
Jigjiga	JJU	600 km E	1600	Semi-arid
Kobo	ARARI	590 km N	1470	Warm-moist
Koga	ARARI	665 km NW	1980	Tepid-moist
Shewa Robit	ARARI	210 km N	1280	Semi-arid
Werer	EIAR	280 km NE	750	Warm-arid

^aInstitutes: ARARI Amhara Regional Agricultural Research Institute, EIAR Ethiopian Institute Agricultural Research, GARI Gambella Agricultural Research Institute, JJU Jigjiga University, SARI South Agricultural Research Institute, TARI Tigray Agricultural Research Institute

MicroRNA

MicroRNA sequencing was done for roots and leaves of two tef cultivars, one drought susceptible (*Alba*) and one drought tolerant (*Tsedey*), under drought and control conditions. Comparison of differentially regulated miRNAs from the two cultivars revealed several homologs of known miRNAs similarly regulated in both cultivars and a few that were differentially regulated and may contribute to differences in drought tolerance. Prediction of the targets of these miRNAs resulted in the identification of many transcription factors that may be potential targets for improvement of drought resistance (Federico Martignelli, personal communication). In addition, a total of 147 novel and unique putative miRNA precursors with mature miRNA sequences were identified.

Proteomics

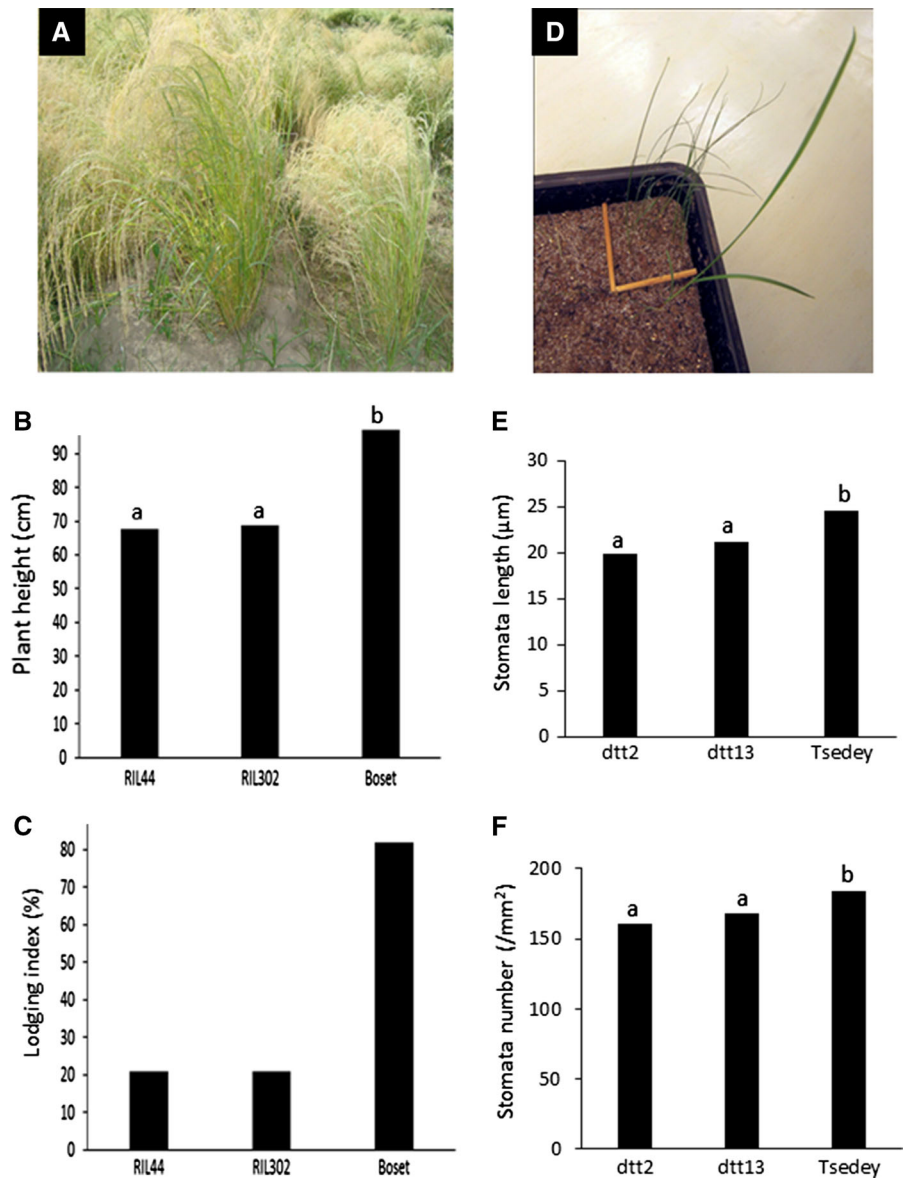
Proteins involved in the biotic and abiotic stress responses, signaling, transport, cellular homeostasis and pentose metabolic processes were found to be more abundant during drought while proteins involved in ROS producing processes such as photosynthetic reactions, cell wall catabolism, manganese transport

and homeostasis, the synthesis of sugars and cell wall modification were less abundant (Kamies et al. 2017). This is the first reported proteomic study of tef.

Release and acceptance of improved tef varieties

For the scaling-up of improved tef technologies to farmers, ten lead farmers were selected in 2015 based on their technical expertise and their level of technology acceptance. These lead farmers represented three districts (namely, Ada, Minjar-Shenkora and Moretna-Jirru) in the Central Highlands of Ethiopia where tef is the major crop (Bekele et al. 2017). In 2016, the number of lead farmers participating in the scaling-up technology increased to 45 from four districts. Participating farmers had a high acceptance of the improved technologies especially the new seeds. Other farmers in the respective communities were also invited to observe the performance of new tef varieties managed by the lead farmers and requests to participate in next year's scaling-up of the project are very high. Although this initial phase of scaling-up involved a limited number of farmers, the improved seeds from the currently participating 45 lead farmers could be distributed to 10,000–15,000 farmers in the coming

Fig. 6 Major achievements of the Tef Improvement Project. **a–c** lodging-tolerant and high yielding tef varieties; **d–f** drought-tolerant tef (*dt*). **a** semi-dwarf and lodging-tolerant variety (*right*) compared to the original line (*left*). Plant height (**b**) and lodging index (**c**) of the two candidate lines, RIL44 and RIL302, compared to the local variety Boset. **d** *dt* (*right*) and original (*left*) lines under moisture scare condition. Stomata number (**e**) and stomata length (**f**) of the adaxial or upper side of the two *dt* lines and the original *Tsedey* variety. Values with the same letter are not significantly different ($p < 0.05$) from each other using a Mann–Whitney test



cropping season. The main reason for the high number of farmers able to participate is the small amount of seed required for sowing tef (as small as 5 kg ha^{-1}) and small farm size per household (0.25–0.5 ha).

Partnerships

The partnership with the Ethiopian Agricultural Research System is critical to the success of our project since it ensures the release and distribution of improved seeds to the subsistence tef farmers in

Ethiopia. Field testing at the on-station and on-farm sites is continuing and the best performing cultivars will be released to farmers after evaluation by the Ethiopian National Variety Release Committee. Financial and technical support from the Syngenta Foundation for Sustainable Agriculture and hosting by the Institute of Plant Science at the University of Bern are the cornerstones of the project. Interest in the program from other research institutions and the media helps to raise the profile of tef research and generate momentum. In general, the collaboration established by the Tef Improvement Project involves a

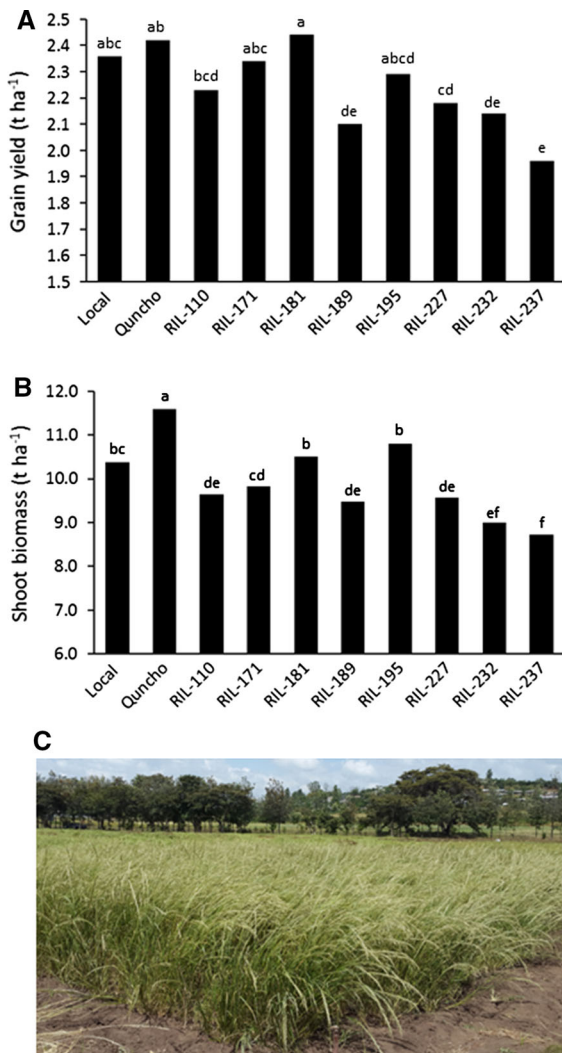


Fig. 7 The performance of several tef varieties including a new line (RIL-181 or *Tesfa*) approved for release by the National Variety Release Committee in Ethiopia. Average grain yield (a) and shoot biomass (b) of 10 lines including eight candidate RILs and the widely cultivated *Quncho* variety tested at six field sites in Ethiopia. The sites were Adet, Debre Zeit black *Vertisols*, Debre Zeit light soil, Chefe-Donsa, Holetta and Ginchi. RIL lines were tested for more than eight generations after crossing to the improved variety or to the local cultivar. RILs investigated were selected from the following crosses: mutant line *kinde* with *Kay muri* (RILs 110, 181, 227, 232 and 237), mutant line *kinde* with *Quncho* (RILs 171 and 189), and mutant line *kegne* with *Kay murri* (RIL 195). Means followed by same letter were not significantly different from each other ($p < 0.05$, ANOVA). c RIL-181 (*Tesfa*) grown in the field at Debre Zeit in black *Vertisols*

prototype of a private–public partnership (PPP). The involvement of farmers at early selection stages is important in order to breed towards the preferences of

end-users (i.e. farmers and consumers). Once the new varieties are approved for release, seed multiplication involves public-, public–private enterprises, and/or privately owned seed companies. In addition, model farmers with know-how in the handling of improved seeds are becoming more involved in multiplying and disseminating the seeds to other farmers.

Globally, the dissemination of improved technologies to farmers in developing countries have been successfully made for locally important crops. Among these, the contributions of the Generation Challenge Programme (GCP) and its close associate Integrated Breeding Platform (IBP) are worthwhile to mention. GCP is a research initiative with more than 200 partners in 54 countries which focuses on exploring plant genetic diversity and developing crops with improved stress tolerance (Frison and Demers 2014; GCP 2017). The major crops of GCP’s focus are root and tuber (cassava), legumes (beans, chickpea, cowpea, pigeon pea and groundnut) and cereals (maize, rice, sorghum and wheat).

On the other hand, IBP is a service provider established by GCP to closely work with plant breeders in developing countries. The platform facilitates the implementation of various stages of a breeding programme by serving as a vehicle for dissemination of knowledge and technology, enabling access to breeding materials and providing cost-effective high-throughput laboratory services (IBP 2017). The IBP aims to increase the effectiveness and efficiency of up to 500 plant breeding programmes across the world in 5 years with a specific focus on smallholder farmers in the developing world (IBP 2017).

Outlook and prospects

Food security is the main concern in Africa especially in Ethiopia, as the production and productivity of crops are under continuous threat (Nichola 2006). Indigenous crops can contribute significantly to food security under the present scenario of increasing world population and changing climate (Naylor et al. 2004). These crops play key roles in the livelihood of smallholder farmers and consumers in Africa since they outperform the major world crops in terms of stress tolerance under the extreme soil and climate conditions prevalent on the continent (Sanginga et al. 2000;

Table 5 SSR (microsatellite) markers present in the tef genome indicating summary statistics and size distribution

Summary statistics		
Total number of sequences examined		405,558
Total genome size (bp)		672,766,097
Total number of identified SSRs		162,124
Number of scaffolds containing SSR		23,431
Number of scaffolds containing more than one SSR		9024
Repeat length	No of required repeats	No of repeats in tef genome
1	10	110,513
2	6	27,880
3	5	19,116
4	5	2008
5	4	2179
6	4	428

Campell 1997; Brandt 1997; Alemaw and Teklewold 1995). Unlike the introduced global crops, these indigenous crops have the major advantage that they fit well into the general socio-economic and ecological context of the region (Tadele 2009; Tadele and Assefa 2012).

The immediate goal of the Tef Improvement Project is to improve crop productivity by tackling major yield limiting factors using state-of-the-art methodology. The ultimate objective, however, is to improve the livelihood of small-scale farmers and consumers in Ethiopia through ensuring food and nutrition security in a country where persistent food insecurity remains a real and major challenge. Hence, the Project will contribute to Ethiopia's economic development through increasing the productivity of the country's most important crop.

We recently (March 2017) reached a milestone—the first release of a cultivar produced in the Tef Improvement Project to Ethiopian consumers. The new release, *Tesfa*, a cultivar developed by crossing an existing cultivar to a candidate discovered through phenotypic screening of a mutagenized population. Many other candidates are progressing through the pipeline and should be released in the coming years. Cultivars tolerant to soil acidity (especially aluminum toxicity), salinity, and non-selective herbicide are in the pipeline at various stages of laboratory research, field testing and crossing.

The overall success of the project depends on the scale of dissemination and adoption of the new cultivars and for this purpose, the importance of a

close working relationships between the University of Bern and the National Agricultural Research System of Ethiopia cannot be overstated.

The increase in productivity and production of tef will substantially reduce if not eliminate the import of grains. Ethiopia annually imports large amounts of both cereal grain and flour. For instance, in the year 2013, the country imported 1.7 million tons of wheat, sorghum and maize at the cost of 712 million USD (FAOSTAT 2015). In order to develop improved crops in general (and tef specifically) for the prevailing and expected challenging environment, we suggest to improve productivity through investing in research and development.

Conclusions

This paper describes a collaborative effort between scientists in Europe and Africa to implement a pipeline for the development of new tef cultivars using both traditional and molecular breeding and to make these novel varieties available to the Ethiopian farmers. After testing under controlled conditions, the generated technology (i.e. the lines with the desirable traits) are transferred to the Ethiopian Institute of Agricultural Research (EIAR) for introgression and field testing. Each candidate line undergoes vigorous field testing at various locations and under different conditions. This cooperation is critical to the success of the project since it ensures the release and distribution of improved seeds to the subsistence farmers in

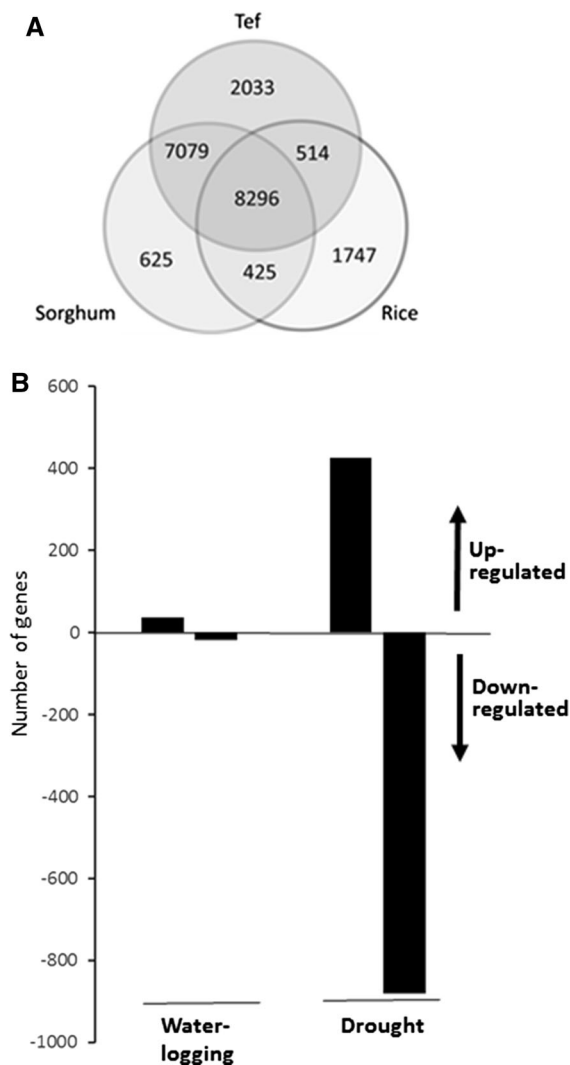


Fig. 8 Genome-scale expression of tef genes. **a** The number of orthologous groups of genes between tef, sorghum, and rice. **b** the number of differentially regulated tef genes under drought and waterlogging conditions

Ethiopia. So far, several semi-dwarf candidate lines with lodging-tolerance and drought-tolerance obtained from the screenings are being introgressed and evaluated in Ethiopia. Farmers are also involved in the selection procedure starting from the on-station research until the release of the improved variety in so-called Farmer Participatory Research. Now that the pipeline has been established and works successfully, further desirable traits such as soil acidity tolerance, increased seed size and improved grain quality are being addressed and the strategy applied to other orphan crops. With improved assembly and annotation

of the tef genome sequence, it will be possible to harness the rich natural variation present in the tef germplasm collections using appropriate techniques. The long-term commitment by the private funding organization is also key to the success of the project. The combination of substantial private funding with a farmer-participatory approach in breeding is a particular merit of the Tef Improvement Project which deserves attention for future initiatives.

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