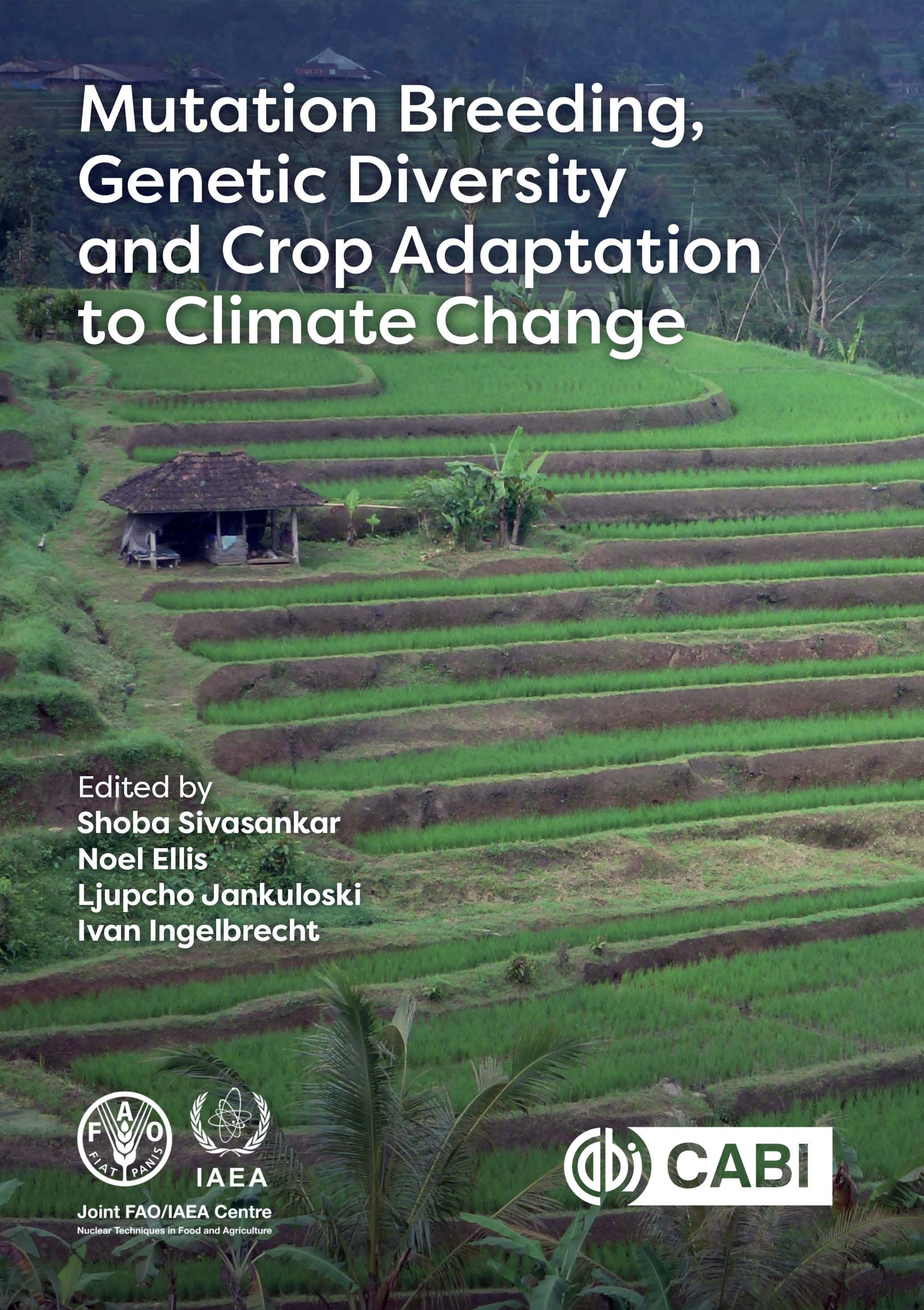

Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change

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Nuclear Techniques in Food and Agriculture
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Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change

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14 Application of Mutation Breeding to the Improvement of the Under-studied Crop Tef (*Eragrostis tef* (Zucc.) Trotter)

Zerihun Tadele[1], Kebebew Assefa, Solomon Chanyalew, Abate Bekele, Annett Weichert, Mirjam Schnell, Nora Röckel, Negussu Hussein and Gina Cannarozzi

Abstract

Induced mutation has been playing a significant role in the improvement of diverse crop types. This led to the release of over 3200 crop varieties in over 70 countries. We implemented induced mutation on tef (*Eragrostis tef* (Zucc.) Trotter), one of 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. Although tef is extensively cultivated in Ethiopia due to its resilience to diverse environmental stresses, the productivity of the crop is very low. The Tef Improvement Project based at the University of Bern in Switzerland employs mutation breeding to tackle major constraints in tef in order to enhance crop productivity. About 12,000 EMS (ethyl methanesulfonate) mutagenized M_2 families were generated from four improved tef varieties, namely 'Tsedey', 'Dukem', 'Kora' and 'Dagim'. Screening for major traits of importance helped us to obtain several candidate lines, including semi-dwarf and lodging-tolerant, drought-tolerant and acid-soil-tolerant lines. Among these, the most promising ones were introgressed to locally adapted improved varieties followed by several years of testing at representative locations for traits of interest. As a result, a new variety called 'Tesfa' with a novel and desirable combination of traits was approved for release to the farming community. This shows that the project has been actively involved in all three phases of induced mutation: mutation induction, mutation detection and mutation breeding.

Keywords: EMS • *Eragrostis tef* • mutation breeding • mutation detection • mutation induction

1 Role of Induced Mutation in Crop Improvement

Crop production is under continuous challenge from diverse environmental constraints, which include a variety of biotic and abiotic stresses, as well as policy-related constraints. The urgency of boosting crop productivity at the present time is due to: (i) the higher rate of population growth compared with the increase in food production; (ii) the widespread problem of biotic and abiotic stresses; (iii) weakness in the inherent properties of the plant (e.g. susceptibility of the plant to lodging); (iv) the cultivation of plants for biofuel production at the expense of food crops; (v) shortcomings in land and investment policies; and (vi) the negative impact of climate change on crop production. Although multiple challenges exist, boosting productivity of crops per unit area is important as it narrows

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down the wide yield gap that exists between the potential and current yield for most crops, especially in the developing world (Tadele, 2017).

Crop improvement has long been dependent on harnessing the naturally existing huge diversity for the trait of interest. However, the required level of diversity might not exist in landraces for some key agronomic traits. This might be due to the tight genetic linkage between the trait of interest and other undesirable traits. It is extremely difficult, if not impossible, to separate closely linked traits using conventional crossing and selection methods of plant breeding.

Induced mutation is, however, considered powerful as it can randomly mutate any trait of interest. In addition to creating variability from which breeders can select for any trait of their choice, induced mutation can also precisely knock out a single gene from a pair of tightly linked ones. Hence, the problem of linkage drag can be minimized.

Induced mutation began about seven decades ago to improve the productivity and/or quality of plants, as it creates stable and heritable alterations in the genetic material of the organism. Since then, mutation breeding has contributed significantly to the improvement of many economically important crops. Crops descended from this technique were superior to the original cultivars in productivity and/or tolerance to biotic and abiotic stresses. The list of officially released and commercially available crop varieties that originated from induced mutation is available in the Mutant Variety Database (MVD) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (IAEA, 2018). The majority of the 3281 varieties recorded so far are from Asia (60%) and Europe (30%) (Table 14.1). Asian countries such as China, Japan and India together with European countries such as Russia and Germany have invested significantly in mutation breeding and have released a large number of varieties of food crops. An exception to this is The Netherlands, where the majority of mutant varieties released have been ornamental rather than food crops. In Africa and Latin America, on the contrary, not enough emphasis has been given to mutation breeding as yet and only a few varieties have been released using this technique. Of all the globally released crop varieties, about 50% are cereals, including rice, wheat and barley. Some of these varieties have been playing a significant role in the economy of some countries in providing substantial contributions to food, feed, fibre and brewing.

2 Tef as a Crop of Choice for Induced Mutation

Tef (*Eragrostis tef* (Zucc.) Trotter) is the most important cereal crop in the Horn of Africa, especially in Ethiopia, where it is annually cultivated on over 3 million hectares of land, which is equivalent to 30% of the total area allocated to cereals (CSA, 2014). The crop is preferred both by farmers and by consumers. Farmers prefer cultivating tef to other cereals since tef is more resilient to environmental stresses such as poor soil drainage during the rainy season and also to moisture scarcity. In addition, as a cash crop, both the grain and the straw of tef fetch a higher price than 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), and the absence of gluten (Spaenij-Dekking *et al.*, 2005) which makes it an alternative food for people suffering from celiac disease. In general, tef plays a vital role in food security, nutrition and income generation to smallholder farmers.

Despite its versatility in adapting to extreme environmental conditions, the productivity of tef is very low in Ethiopia at 1.5 t/ha, compared with 3.2 t/ha for maize (CSA, 2014). The major yield-limiting factors are lack of cultivars tolerant to lodging and drought (Assefa *et al.*, 2011), as well as small seed size. This 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 bottleneck in tef production. 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 crop husbandry, as the application of increased amounts of nitrogen fertilizer to boost the yield results in severe lodging. Consequently, both the yield and the quality of the grain and the straw are severely reduced. The lodged plant also poses difficulties in harvesting.

Table 14.1. The number and type of crop varieties released from induced mutation in different continents and countries. Major food crops that benefited from these programs are indicated. Adapted from IAEA (2018).

Continent	Varieties released	Top countries	Varieties released	Major food crops
Asia	1993	China	810	Rice, wheat, soybean, maize
		Japan	479	Rice
		India	335	Rice, barley, peanut
		Bangladesh	70	Lentil, chickpea, peanut, rice
		Pakistan	59	Mung bean, rice, wheat, chickpea
		Vietnam	58	Rice, soybean
Europe	995	Russia	216	Barley, buckwheat, wheat
		Netherlands	176	No food crop
		Germany	171	Barley
		France	39	Barley, rice
		Italy	35	Durum wheat
		UK	34	Barley
North America	200	USA	139	Barley, bean, rice
		Canada	40	Beans
Africa	72	Côte d'Ivoire	25	Rice
		Mali	15	Rice, sorghum
Latin America	51	Guyana	26	Rice
		Brazil	14	Bean
Australia & Pacific	10	Australia	9	Oats

The National Tef Improvement Program at Debre Zeit Agricultural Research Centre of the Ethiopian Institute of Agricultural Research began using induced mutation techniques in the early 1970s, specifically in 1972 with technical support from the IAEA. The method was then considered as the only option for creating variability in tef, as it was believed that tef flowers were entirely cleistogamous and not amenable to cross-breeding. This belief was later proven to be wrong after it was discovered that the cleistogamous nature of tef florets is incomplete and that they open early in the morning. This discovery eventually enabled the development of the artificial tef hybridization technique by Tareke Berhe in 1974 (Berhe, 1975). In the early efforts, through the technical support of the IAEA, a gamma-ray irradiation facility (Gamma Cell 220) was established at Debre Zeit. In addition, there is an ongoing project on induced mutation at the centre. In all the efforts, the major traits of interest were improvement of lodging tolerance, resistance to leaf rust disease (*Uromyces eragrostidis* Tracy) and resistance to shattering. In spite of repeated attempts made over the years, however, no tef varieties have been released through the conventional induced mutation techniques employed.

The Tef Improvement Project at the University of Bern in Switzerland was established a decade ago to boost the productivity of tef by tackling major production constraints through developing cultivars with desirable agronomic and nutritional traits. This project focuses on problem-oriented and demand-driven research. Priority has been given to developing cultivars with resistance to lodging and drought tolerance, since these two constraints contribute towards significant yield losses in tef (Assefa *et al.*, 2011).

The strategy and pipeline to develop new cultivars with valuable agronomic traits have been presented previously (Cannarozzi *et al.*, 2018). The three phases of the project are technology generation, technology transfer and technology delivery. Under the *Technology Generation* phase, modern genetic, molecular and genomic tools are applied to obtain candidate tef lines for traits of interest. In the *Technology*

Transfer phase, promising tef lines harbouring 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. In the *Technology Delivery* phase, seeds of newly released varieties are multiplied and disseminated through private and public institutions.

There are over 5000 tef landraces collected and conserved at the Ethiopian Biodiversity Institute. Although the entire germplasm has not yet been thoroughly evaluated, huge diversity was reported and some of these accessions were very distinct as assessed by morphological and agronomic traits (Chanyalew *et al.*, 2013; Plaza-Wüthrich *et al.*, 2013; Assefa *et al.*, 2015; Jifar *et al.*, 2015). However, this substantial phenotypic diversity had not enabled us to identify lodging-tolerant tef lines.

As a result, the Tef Improvement Project embarked on a large-scale mutation breeding project.

2.1 Mutation induction in tef

Mutation induction, which refers to the creation of genetic diversity, can be investigated based on at least four aspects: (i) source of mutation (natural or induced); (ii) type of mutagen (physical or chemical); (iii) patterns of DNA cleavage intensity of mutation (point mutation, indels or rearrangements) and spectrum of mutation (nonsense, missense, silent or splice junction); and (iv) precision of the mutation (random or targeted) (Tadele, 2016). In order to establish mutagenized populations, seeds of four improved tef varieties, namely, 'Tsedey' (DZ-Cr-37), 'Dukem' (DZ-01-974), 'Kora' (DZ-Cr-438 RIL-133B) and 'Dagim' (DZ-Cr-438 RIL91A), were used for mutagenesis. A widely applied chemical mutagen, ethyl methanesulfonate (EMS), was used to treat tef seeds (Sikora *et al.*, 2011; Mba, 2013). EMS mainly creates point mutations (G to A transition) in which a single nucleotide is altered. Before embarking on large-scale mutagenesis, the optimum level of EMS was determined (Fig. 14.1). Based on the germination and growth of treated plants, treatment with 0.2%

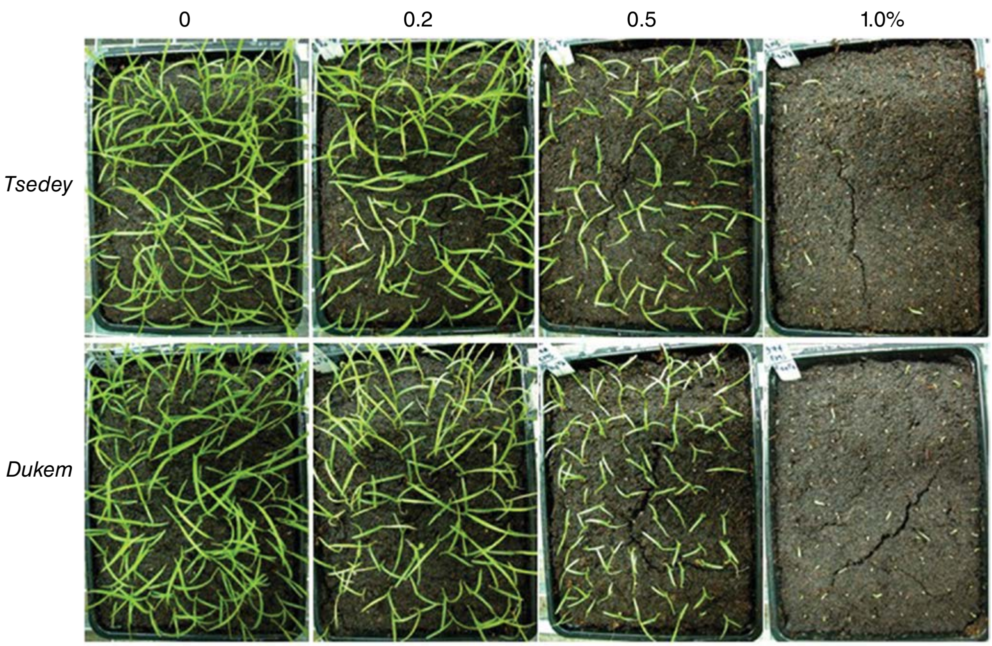

Fig. 14.1. Effect of different concentrations of ethyl methanesulfonate (EMS) on germination and seedlings of two tef varieties ('Tsedey' and 'Dukem'). Seeds were treated for 8 h each with four concentrations of EMS shown as %v/v at the top of the figure.

(v/v) EMS for 8 h was selected for large-scale mutagenesis of tef seeds.

Approximately 20,000 seeds from the four elite cultivars treated with EMS as above were grown as individual M_1 plants to set seeds. About 12,000 M_2 families derived from these M_1 plants have been used in the screening for diverse trait(s) of interest (Table 14.2) (Fig. 14.2).

2.2 Mutation detection in tef

Mutation detection refers to the discovery of either the altered gene or the mutant line. In forward genetics, the genes altered in the candidate lines are elucidated, whereas in reverse genetics, mutant lines with defects in the genes of interest are identified. We applied both approaches for the discovery of either the gene or the mutant line.

In the forward genetics approach, the mutagenized tef population was used for phenotypic screening to obtain candidate mutant lines for the traits of interest. Moreover, the same populations were used in reverse genetics approaches: TILLING (Targeting Induced Local Lesions in Genomes) to screen for mutant lines that harbour DNA lesions in genes of interest. TILLING is a non-transgenic method and it has been applied to several crops, including major and orphan crops (Tadele *et al*., 2010; Esfeld *et al*., 2013). Some benefits of TILLING are as follows.

1. It produces a spectrum of allelic mutations that are useful for genetic analysis.
2. It reveals mutations that are difficult to identify by forward genetics, since TILLING can focus on a particular gene of interest.
3. It applies to any organism regardless of genome size and ploidy level.
4. It produces stable mutations.

Table 14.2. The number of mutagenized tef populations from diverse background genotypes and their current status.

Background genotype				
Common name	Variety name	No. of M_2 populations	Traits screened for	Current status
Tsedey	DZ-Cr-37	5000	Diverse agronomic traits	Different stages
Dukem	DZ-01-974	2000	Drought tolerance	Inbred line testing at multi-locations
Kora	DZ-Cr-438 RIL133B	2500	Diverse agronomic traits	Different stages
Dagim	DZ-Cr-438 RIL91A	2400	To be determined	Planning phase

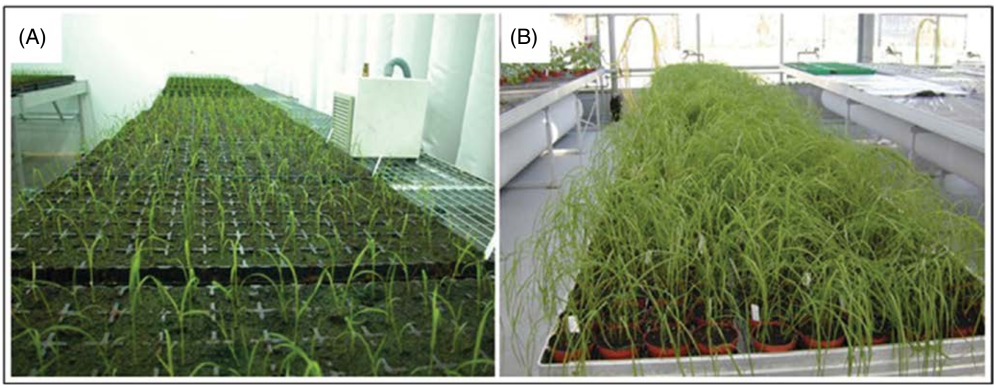

Fig. 14.2. Partial view of large-scale cultivation of mutagenized tef plants in the greenhouse. **(A)** M_1 individual lines. **(B)** M_2 populations derived from individual M_1 lines.

5. Since no exogenous DNA is introduced into the plant by the chemical mutagenesis, the product is considered non-transgenic and is exempted from regulatory procedures imposed on transgenic products (Tadele *et al*., 2010; Alonso and Ecker, 2006).

The technique of TILLING comprises the following main steps: (i) mutagenesis; (ii) development of a non-chimeric population; (iii) preparation of a germplasm stock; (iv) DNA extraction and sample pooling; (v) screening M_2 families to detect mutations in the desired gene; and (vi) identification of mutant lines and sequencing of target gene (Till *et al*., 2006). As indicated earlier (Tadele *et al*., 2010; Alonso and Ecker, 2006), TILLING has been applied in M_2 mutagenized tef families. Focus has been given to identifying mutant lines altered in plant height and starch type or content. The TILLING project has benefited immensely from the Tef Genome Sequencing Project, especially in designing unique primers for the allotetraploid tef with two very close genomes.

Phenotypic screenings for the traits of interest were done mainly at the University of Bern. A partial list of promising candidate mutant lines obtained from diverse screenings is shown in Table 14.3. Emphasis was given to screening for semi-dwarf lines, since semi-dwarfism during the Green Revolution in wheat and rice has been associated with lodging tolerance (Peng *et al*., 1999; Spielmeyer *et al*., 2002; Hedden, 2003).

Kegne was the first semi-dwarf candidate line obtained through phenotypic screening of over 5000 mutagenized M_2 families. In addition to semi-dwarfism and lodging tolerance, *kegne* plants have unique properties. Among these, one is the twisting of the leaves and stem of the plant

Table 14.3. Selected candidate lines from EMS mutagenized tef populations screened for traits of interest.

Candidate line	Background genotype	Desirable trait	Screening institution	References
Kegne	*Tsedey*	Semi-dwarf	University of Bern	Jost *et al*. (2015)
Kinde	*Tsedey*	Semi-dwarf	University of Bern	Cannarozzi *et al*. (2018)
dtt2	*Tsedey*	Early drought tolerance	University of Bern	Schneider (2011)
dtt13	*Tsedey*	Early drought tolerance	University of Bern	Schneider (2011)
tdt4-5	*Dukem*	Terminal drought tolerance	University of Bern	Rindisbacher (2015)
tdt4-15	*Dukem*	Terminal drought tolerance	University of Bern	Rindisbacher (2015)
tdt4-19	*Dukem*	Terminal drought tolerance	University of Bern	Rindisbacher (2015)
ml-209	*Tsedey*	Acid soil tolerance	Kwazu Natal – ARARI	Desta *et al*. (2017)
ml-153	*Tsedey*	Acid soil tolerance	Kwazu Natal – ARARI	Desta *et al*. (2017)
meten 1	*Kora*	Semi-dwarf	University of Bern	This work
meten 2	*Kora*	Semi-dwarf	University of Bern	This work
meten 3	*Kora*	Semi-dwarf	University of Bern	This work
Several candidates	*Tsedey*	Starch altered	ETH Zurich	W. Wang (personal communication)
sde (semi-dwarf & early)	*Kora*	Semi-dwarf and early maturing	University of Bern	This work
Several	*Kora*	Salinity tolerance	University of Bern	This work
Several	*Kora*	Acid soil tolerance	University of Bern	This work

to the right side (Jost *et al*., 2015). This twisting in *kegne* plants (about 12°) is similar to *twisted dwarf 1* (*tid1*) in rice (Sunohara *et al*., 2009) and *spr1* or *spr2* in Arabidopsis (Furutani *et al*., 2000). The candidate gene approach allowed us to identify the microtubule-associated gene affected in the *kegne* mutant and also to design the Cleaved Amplified Polymorphic Sequences (CAPS) marker to trace the mutant after crossing to other tef genotypes (Jost *et al*., 2015).

The other promising semi-dwarf line obtained from phenotypic screening was *kinde* (Fig. 14.3). This line possesses a number of desirable properties, including higher tillering number and increased lodging tolerance.

Screening for drought tolerance targeted both early drought, which occurs during the onset of the growing season, and terminal drought, which occurs during the flowering or maturity period of tef plants. Mutations in two candidate genes for early drought tolerance named *drought tolerant tef* (*dtt2* and *dtt13*) tolerate about 3 weeks of moisture scarcity, while the original tef lines could not withstand this level of drought (Schneider, 2011). Both *dtt* lines have fewer and smaller stomata compared with the original line and these might limit the loss of water through transpiration.

Screening for terminal drought-tolerant lines also resulted in three candidate lines: *tdt4-5*, *tdt4-15* and *tdt4-19* (Rindisbacher, 2015).

3 Mutation Breeding

Candidate tef lines from diverse screening programmes were sent to the National Tef Research Program in Ethiopia where they were hybridized to locally adapted and high-yielding varieties. Due to their desirable traits, candidate mutant lines were used as parental lines in over 50 crosses. A partial list of the crossings and current status is shown in Table 14.4. Each cross generated about 500 F_2 lines from which selection has been made for the trait of interest. While some of these crosses are at an early breeding stage, others are at an advanced stage where they are undergoing multi-location testing at representative sites across the country (Table 14.5). In general, these breeding programmes correspond to 16 experiments under early and late maturity groups. While germplasm in the early-maturity groups is tested at semi-arid locations with moisture deficit, those with late maturity are evaluated in areas with adequate precipitation.

After years of multi-location testing, the new variety called 'Tesfa' was approved for release by the National Variety Release Committee in Ethiopia. The variety 'Tesfa' has the following desirable traits: compact panicle, tolerance to lodging, non-shattering and thick culm making it suitable under irrigated conditions (Kebede *et al*., 2018) (Fig. 14.4). Currently, this newly released 'Tesfa' is being

Fig. 14.3. The semi-dwarf and lodging tolerant *kinde* line (left) obtained from screening a mutagenized population in the background of 'Tsedey' cultivar (right). Bar = 10 cm.

Table 14.4. A selection of crosses made to candidate mutant tef lines developed by the Tef Improvement Project and their current status.

Mutant line		Crossed to		
Name	Desirable trait	Name	Desirable trait	Current status[a]
Kegne	Lodging-tolerant	*Key Murri*	High culm strength	VVT
		Magna	White-seeded variety	NVT
		Quncho	Popular variety	NVT
		Tsedey	Drought tolerant	F_5 seeds
Kinde	Lodging-tolerant	*Alba*	Early maturing	VVT
		Boset	Drought tolerant	F_4 seeds
		Dukem	High yielding	F_4 seeds
		Key Murri	High culm strength	NVT
		Kora	High yielding	F_4 seeds
		Magna	White-seeded	F_4 seeds
		Quncho	Popular variety	NVT
		Tsedey	Drought tolerant	F_5 seeds
dtt2	Drought-tolerant	*dtt13*	Drought tolerant	OBN
		Kegne	Semi-dwarf	F_5 seeds
		Key Murri	High culm strength	PVT
		Magna	White-seeded	F_4 seeds
		Quncho	Popular variety	OBN
dtt13	Drought-tolerant	*Boset*	Drought tolerant	F_4 seeds
		dtt2	Drought tolerant	OBN
		Key Murri	High culm strength	F_4 seeds
		Magna	White-seeded	F_4 seeds
tdt4-15	Terminal drought tolerance	*Dukem*	High yielding	F_3 seeds
		Kora	High yielding	F_3 seeds
		Magna	White-seeded	F_4 seeds
tdt4-19	Terminal drought tolerance	*Boset*	Drought tolerant	F_3 seeds
		Dukem	High yielding	F_3 seeds
		Kora	High yielding	F_3 seeds
		RIL44	Semi-dwarf	F_3 seeds

[a]NVT, National Variety Trial; OBN, Observation Nursery; PVT, Preliminary Variety Trial; VVT, Variety Verification Trial

outscaled in four districts in central Ethiopia together with other improved technologies (Bekele *et al.*, 2017).

4 Discussion

The goal of this manuscript is to show the highlights of our initiative and the progress made in mutation breeding on tef.

5 Conclusion

Mutation breeding is the cornerstone of the Tef Improvement Project based in Bern, Switzerland, which closely collaborates with the National Tef Improvement Program in Ethiopia. Since the project focuses on problem-oriented research by tackling major constraints affecting tef productivity, emphasis has been given to developing lodging and drought-tolerant cultivars. Candidate mutant lines identified in Bern were sent to Ethiopia, where they were incorporated into the national breeding programme. 'Tesfa', the first tef variety released from the mutation background, has received high acceptance by smallholder farmers. In general, our value-chain approach which starts from the basic research of identifying candidate mutant lines to the breeding and dissemination of improved technology relied on mutagenized populations.

Table 14.5. Locations in Ethiopia where germplasm from the Tef Improvement Project have been evaluated after crossing to improved tef varieties.

Centre/site	Geographical coordinates	Distance and direction from Addis Ababa	Altitude (m asl)	Climate
EIAR (Ethiopian Institute of Agricultural Research)				
Adadi Mariam	8°37′N, 38°30′E	55 km S	1900	Sub-humid
Akaki	8°53′N, 38°47′E	10 km S	2300	Cool-wet
Alem Tena	8°18′N, 38°57′E	110 km S	1650	Semi-arid
Ambo	8°59′N, 37°51′E	115 km W	2185	Temperate
Asosa	10°04′N, 34°31′E	655 km W	1590	Warm to sub-humid
Chefe Donsa	7°32′N, 40°38′E	40 km SE	2400	Cool-wet
Debre Zeit-black soil	8°44′N, 39°00′E	45 km S	1800	Temperate
Debre Zeit-light soil	8°44′N, 39°00′E	45 km S	1800	Temperate
Dhera	7°44′N, 39°29′E	122 km SE	1680	Semi-arid
Ginchi	8°54′N, 38°09′E	90 km W	2200	Tepid-moist
Holetta	9°03′N, 38°30′E	35 km W	2390	Cool-wet
Jimma	7°40′N, 36°50′E	365 km SW	1760	Sub-humid
Melkassa	8°24′N, 39°21′E	115 km SE	1550	Semi-arid
Minjar	9°09′N, 39°19′E	110 km SE	1800	Semi-arid to moist
Pawe	11°19′N, 36°19′E	575 km NW	1000	Sub-humid
Werer	9°16′N, 40°09′E	280 km NE	750	Warm-arid
Wolenchiti	8°40′N, 39°26′E	120 km SE	1400	Semi-arid
ARARI (Amhara Regional Agricultural Research Institute)				
Adet	11°16′N, 37°29′E	445 km NW	2240	Moist-cool
Bichena	10°27′N, 38°12′E	270 km NW	2500	Cool-wet
Kobo	12°09′N 39°38′E	590 km N	1470	Warm-moist
Koga	11°24′N, 37°09′E	665 km NW	1980	Tepid-moist
Metema	12°58′N, 36°12′E	900 km NW	690	Sub-humid
Shewa Robit	10°00′N, 39°54′E	210 km N	1280	Semi-arid
Simada	11°29′N, 38°14′E	770 km NW	2470	Semi-arid
Sirinka	11°45′N, 39°36′E	510 km N	1850	Semi-arid
Oromia (Oromia Agricultural Research Institute)				
Bako	9°06′N, 37°09′E	250 km W	1590	Sub-humid
Shambu	9°40′N, 36°59′E	305 km W	2500	Cool-wet
TARI (Tigray Agricultural Research Institute)				
Axsum	14°16′N, 39°09′E	955 km N	2100	Semi-arid
Humera	14°00′N, 37°00E	975 km NW	600	Warm-moist lowland
Mehoni	12°39′N, 39°44′E	635 km N	2400	Arid
SARI (South Agricultural Research Institute)				
Jinka	5°46′N, 36°33′E	580 km SW	1500	Sub-humid lowland
GARI (Gambella Agricultural Research Institute)				
Abobo	7°49′N, 34°29′E	750 km W	500	Sub-humid
Universities				
Haramaya (Hirna)	9°24′N, 42°01′E	365 km E	1775	Cool-wet
Jigjiga	9°21′N, 42°48′E	600 km E	1600	Semi-arid
Wolkite	8°17′N, 37°47′E	190 km SW	1920	Sub-humid

Fig. 14.4. Mutation breeding allowed us to release the first variety with improved properties. A new tef variety (left) is compared to the standard variety (right) near Debre Zeit. (Photo: Z. Tadele, 5 October 2018.)

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