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Research article

Genetic Diversity of released Tef (*Eragrostis tef (Zucc.) Trotter)* Varieties for Lodging Resistance.

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Genetic improvement of native crops is a new and promising strategy to combat hunger in the developing world. Tef is the major staple food crop for approximately 50 million people in Ethiopia. As an indigenous cereal, it is well adapted to diverse climatic and soil conditions; however, its productivity is extremely low mainly due to susceptibility to lodging. The presence of considerable variations among tef genotypes has been observed indicating the potential of improving the crop through selection of genotypes that combine the traits of interest. The objective of this review: (1) the variation of released tef varieties/germplasm materials for key lodging resistance traits, (2) to assess the impact of lodging on productivity of tef. This review presented that there is positive association between lodging index and grain filling period indicated that the shorter the time to grain filling, might help to reduce lodging of tef as well as the longer grain filling period giving higher grain and causes higher lodging. Therefore, selection of genotypes with short days to grain filling and maximum number of productive tillers reduce the lodging index and increase lodging index, respectively. This shows that lodging index had negative correlation with days to head, days to maturity, plant height and culm length. Tef genotypes that exhibited short days to heading and grain filling period need to be considered as potential genetic materials for improving tef production through selection. A multi-faceted approach involving different disciplines has been suggested to combat the problem of lodging in tef.

Keywords: *Eragrostis tef*, diversity, genotype, lodging, tef, variability

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INTRODUCTION

Even though, tef has numerous merits and considerable economic significance in Ethiopia, the national average grain yield of tef is relatively low (1575 kg ha-1) (CSA, 2015). However, Tareke *et al.* (2013) reported that the tef yields of 4000 and 2500 kg ha-1 on research fields and on farmers' fields, respectively. The major yield limiting factors are the low yield potential of landrace tef, lack of cultivars tolerant to lodging, drought and pests (Kebebew *et al*., 2011).

The presence of diverse genotypes in tef has been reported and it is a good opportunity for breeders to select genotypes for traits of interest. The characters with huge variability include days to maturity (60 to 120 days), plant height (31 to 155 cm), number of tillers/plant (5 to 35) (Seyfu,1993; Kebebew *et al*., 2001). However, it is necessary to investigate further about the magnitude of variation for desirable traits in many genotypes at different locations as much as possible to have good knowledge that can be used to select tef genotypes in breeding programs (Khan *et al.,* 2010; Kotal *et al.,* 2010).

Breeding for lodging tolerance in any given crop, has immense value to the farmers as their livelihood depends on the harvest(s). It bears a positive effect on the farmers' economic health, family well-being and harmony in the society. Thus as a trait, lodging and drought resistance has immense value to the individual farmer and the society. The plant has a wide range of genetic and phenological adaptations innate or triggered to cope with the stress.

Tef, *Eragrostis tef* (Zucc.) Trotter is a member of the grass family Poaceae and genus *Eragrostis.* The genus *Eragrostis* constitutes about 350 species of which only tef is cultivated for human consumption (Watson & Dallwitz, 1992). Tef is an allotetraploid species with a base chromosome number of 10 (2n=4x=40) with genome size of 730 Mbp (Ayele *et al.,* 1996). It is self-pollinated with chasmogamous and hermaphroditic flowers. It has very low degree of out-crossing, that ranges from 0.2% - 1.0% and a C4, plant species (Seyfu, 1997).

The Ethiopian diversified agro-ecology and culture makes the country to be the center of origin and diversity for many economically important crops including tef [Eragrostis tef (Zucc.) Trotter], which belongs to the Grass or Poacea family and the genus Eragrostis. This genus comprises about 350 species of which 54 are found in and 14 are endemic to Ethiopia. Therefore, Tef is believed to have originated and diversified in Ethiopia (Vavilov, 1951).

Tef represents a unique biodiversity component in the agriculture and food security systems of millions of poor farmers in Ethiopia. Its domestication is considered as one of the legacies of Ethiopian farmers to the world (Seyfu, 1997). The crop is grown outside Ethiopia only in few countries including Eritrea, Kenya, Yemen, Malawi, and India. Tef is also grown as a fodder crop in other countries including USA, South Africa and Australia. Since very recently, however, the use of tef as a cereal for humans is exceeding the boundaries of Ethiopia and commercial production has begun in the United States and South Africa, whereas some farmers in Netherlands, Spain, Israel and Australia have also experimented with it (Kebebew *et al.,* 2011). It is considered as the "latest super food of the 21st century" such that its international popularity is rapidly growing (Collyns, 2013).

Tef is produced for different purposes including food and feed, cash and foreign currency earnings. In Ethiopia it is the leading crop accounting for 29.71% of the total acreage and 20.12% of the gross grain production of all cereals grown in the country (CSA, 2015).

Tef is a resilient crop that performs better than other cereals under local conditions including drought, water logging, and poor soil. Since it produces a reasonable yield when grown in areas that experience moisture scarcity, it is considered as a low risk crop (Seyfu, 1997).

Tef 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) makes it an alternative food for people suffering from coeliac disease. Tef is a diverse species grown in different climatic and edaphic zones in Ethiopia, at elevations of 1000 to 2500m. It is considered to be well adapted to drought and waterlogged conditions (Wondewosen, 2012**).**

A major cause of low productivity of tef is lodging, the permanent displacement of the stem from the upright position. Tef has a tall and slender stem which is susceptible to lodging caused by wind and rain.

In addition, when fertilizer is applied to increase yield, stems of tef grow taller and become even more susceptible to lodging, resulting in significantly reduced quantity and quality of grain and straw. Moreover, lodging makes harvesting by hand difficult and mechanical harvesting nearly impossible. The average yield reduction due to lodging is estimated at 17% (Seyfu, 1993).

Despite its versatility in adapting to adverse environmental conditions and being the staple food for ~50 million people in the Horn of Africa, seed yield of tef is low (1.56t/ha CSA 2016), it has very low productivity of tef because of, Drought, lodging.

The objective(s) of this review paper is, therefore, to review: (1) the variation of released tef varieties/germplasm materials for key lodging resistance traits, (2) to assess the impact of lodging on productivity of tef.

LITERATURE REVIEW

Botany of Tef

Tef is a fine stemmed, tufted annual grass characterized by a large crown, many shoots, and a shallow fibrous root system. The plants germinate quickly and are adapted to environments ranging from drought stress to waterlogged soil conditions. The inflorescence is an open panicle and produces small seeds (1,000 weights 0.3 to 0.4 g). The florets consist of a lemma, three stamens, two stigma and two lodicules. Floret colors vary from white to dark brown. Plant height of tef varies from 25–135 cm depending on cultivar type and growing environments. The panicle length, ranges from 11–63 cm and the spikelet numbers per panicle varies from 190-1410. Panicle types also vary from loose, lax, compact, multiple branching, multi-lateral and unilateral loose to compact forms (Hesselbach and Westphal, 1976).

Importance of Tef in Ethiopia

Tef is the most important cereal of Ethiopia (National Tef Commodity Research Strategy (2016-2030)

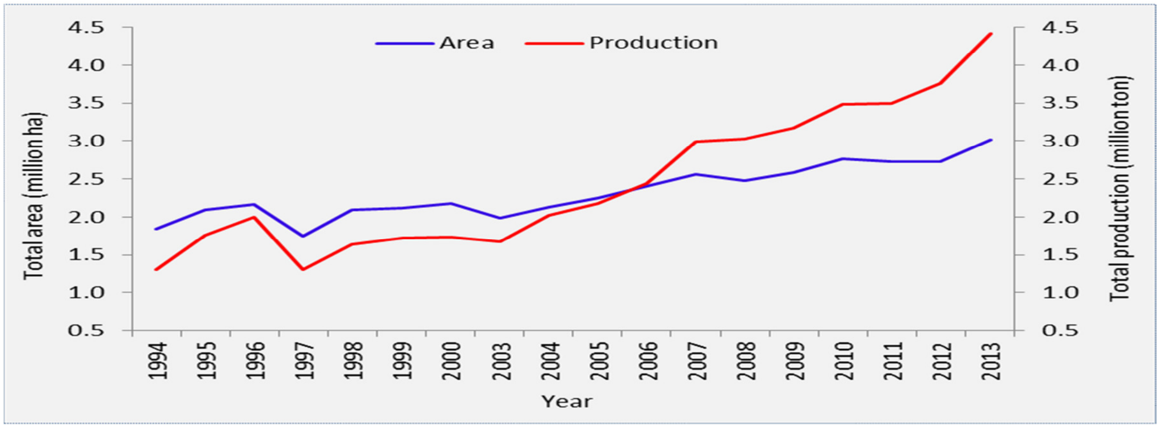


Figure 1: *The total area of cultivation and gross grain production of tef in Ethiopia from 1994 to 2013.*

Table 1. *Major tef producing regions of Ethiopia during the 2013 main season (2005/06 E.C.) (CSA, 2014)*

Region	Area (ha)	% of total cereal area	Production (ton)	% of total cereal production	Yield (ton/ha)
Tigray	185,337	24.43	246,552	16.83	1.33
Amhara	1,146,730	34.13	1,713,797	25.54	1.50
Oromia	1,397,797	30.43	2,104,084	19.22	1.51
SNNPR	259,508	30.02	327,506	17.67	1.26
Total (Ethiopia)	3,016,522	30.62	4,418,642	20.47	1.47

Food and Nutrition

Table 2. *Nutritional composition of tef grains compared to other major world cereals (per 100 g of grains)*

Nutritional Item	White tef	Brown tef	Mixed tef	Finger millets	Barley	Maize	Wheat	Sorghum
Calories (g)	339	336	336	326	334	356	339	338
Moisture (%)	10.4	11.1	10.7	12.1	11.3	12.4	10.8	12.1
Protein (g)	11.1	10.5	8.3	7.2	9.3	8.3	10.3	7.1
Fat (g)	2.4	2.7	2.9	1.4	1.9	4.6	1.9	2.8
Carbohydrates (g)	73.6	73.1	75.2	77.1	75.4	73.4	71.9	76.8
Fiber (g)	3	3.1	3.6	5.6	3.7	2.2	3	2.3
Ash (g)	2.5	3.1	3.0	3.3	2	1.3	1.5	1.6
Calcium (mg)	156	157	140	386	47	6	49	30
Phosphorous (mg)	366	348	368	220	325	276	276	282
Iron (mg)	18.9	58.9	59	85.1	10.2	4.2	7.5	7.8

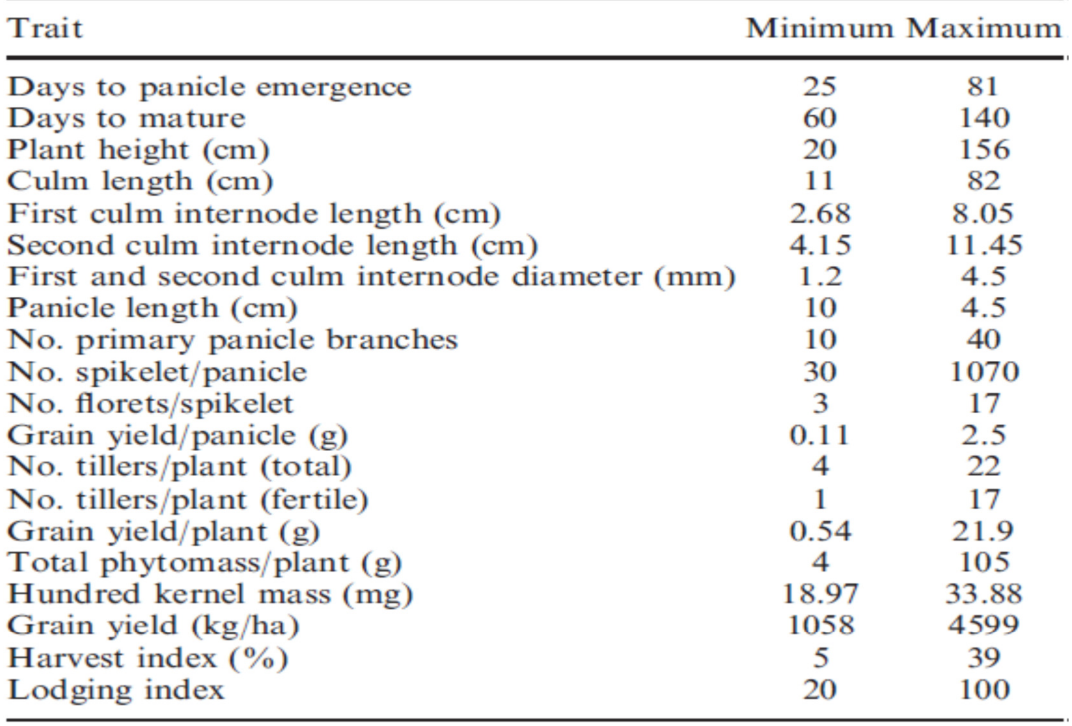
Source: Modified from Agren and Gibson (1968)

Tef genetic resources for breeding

The total numbers of holdings of tef germplasm accessions in the gene bank of the Institute of Bio-diversity Conservation (IBC) in Ethiopia until 2000 was 4395, and of these 1497 accessions were acquired through donations and repatriations and the rest through collections (Demissie, 2001).

The ranges of variation in important phenologic, morphological and agronomic traits of tef obtained based on values reported in different studies are summarized in Table 3. Generally, the species exhibits broad diversity in most of the traits. However, the observed variation for some of the most important traits such as lodging resistance and culm thickness, even when considering the maximum values in the desired direction, is not sufficient enough as to impart a satisfactory level of lodging resistance.

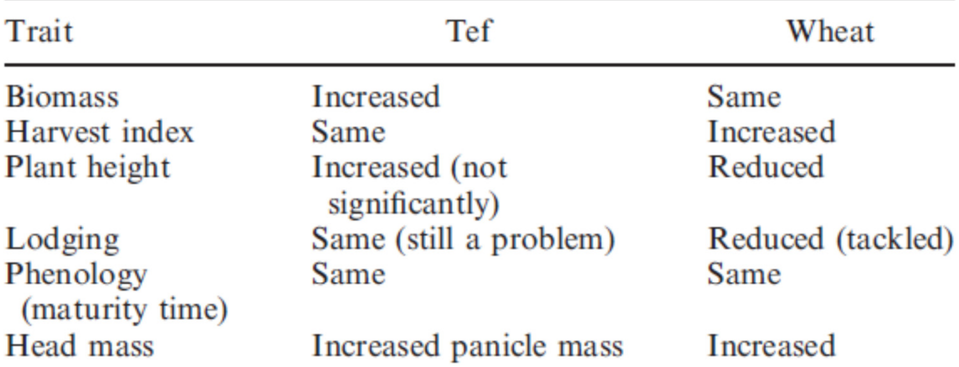
Table 3*: Ranges for important phenologic, morphological and agronomic traits of tef varieties (Source:Kebebew et al. 2001)*



Trait	Minimum	Maximum
Days to panicle emergence	25	81
Days to mature	60	140
Plant height (cm)	20	156
Culm length (cm)	11	82
First culm internode length (cm)	2.68	8.05
Second culm internode length (cm)	4.15	11.45
First and second culm internode diameter (mm)	1.2	4.5
Panicle length (cm)	10	4.5
No. primary panicle branches	10	40
No. spikelet/panicle	30	1070
No. florets/spikelet	3	17
Grain yield/panicle (g)	0.11	2.5
No. tillers/plant (total)	4	22
No. tillers/plant (fertile)	1	17
Grain yield/plant (g)	0.54	21.9
Total phytomass/plant (g)	4	105
Hundred kernel mass (mg)	18.97	33.88
Grain yield (kg/ha)	1058	4599
Harvest index (%)	5	39
Lodging index	20	100

Tef Production Constraints in Ethiopia

Table 4: Challenges in tef traits associated with genetic improvement as Compared to wheat. (Kebebew et al*.,* 2015).



Trait	Tef	Wheat
Biomass	Increased	Same
Harvest index	Same	Increased
Plant height	Increased (not significantly)	Reduced
Lodging	Same (still a problem)	Reduced (tackled)
Phenology (maturity time)	Same	Same
Head mass	Increased panicle mass	Increased

In addition to lodging, Environmental stress is the most important factor which affects crop production. According to Cassman (1999) only about 10% of world arable land may be classified into non-stress category. About 20% of the land is limited by mineral stress, 26% by drought stress and 15% by freezing stress. Modifying the environment for proper crop growth means the alleviation of environmental stresses through the current crop management practices (Arkin and Taylor, 1983). In semi-arid and arid areas, rainfall is inadequate, erratic, and non-uniform in distribution. Moreover, because of degradation and poor vegetation cover, soils in semi-arid and arid areas have low fertility with poor water holding capacity. In addition to the abovementioned problems, weeds also compete with the food crops for the available moisture (Reddy and Kidane, 1991); besides, there are occasional outbreaks of pests and diseases.

What is lodging?

Lodging can be defined as displacement of the aerial parts of the plants (Pinthus, 1973; Seyfu, 1993). It can be induced by both external and internal factors like wind, rain, and morphological traits of the crops or by their interactions. Lodging is among the most important factors threatening increased production and productivity of tef (Seyfu, 1993).The grain bearing organs of cereals are found at the top of the stem, and therefore, exert a strain on the stalk especially under high wind or wind driven rain. Moreover, crop husbandry, crop disease and an abundant supply of nutrients in the soil can contribute to the process of lodging (Tams *et al.,* 2004).

As in other small grain cereals, the tef crop is also prone to severe lodging under favorable and high input husbandry (Fufa *et al.,* 2001; Tekalign *et al.,* 2001). This is ascribed to the morphological features of the plant, hence posing significant losses in quality and quantity of both grain and straw yield.

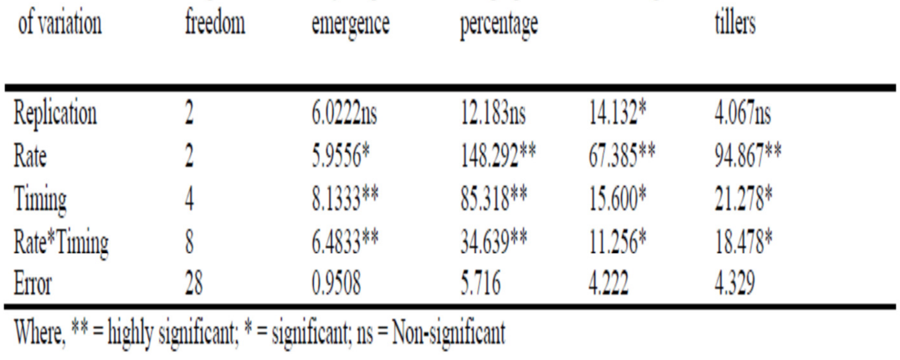
Lodging poses serious economic losses, and also impairs the quality of products, directly or indirectly. Directly, it affects dry matter accumulation, and indirectly, imposing difficulty in harvesting. Bend lodging in tef causes losses in seed and straw, and poses problem during harvesting (Seyfu, 1997). Lodging indirectly prevents the attainment of high grain yield through making mechanized harvesting difficult whereas harvesting by hand as practiced traditionally is time-taking and tiresome. In addition, lodging causes direct loss in tef through affecting important yield components such as thousand seed weight and yield per panicle and it causes damage to the vegetative part of the plant, due to rotting and fast spread of disease and pests (Seyfu, 1993). The overall loss in grain yield due to lodging under natural condition was estimated within the range of 11-22% with an average loss of **17%** (Seyfu, 1983).

The ability of a crop to withstand lodging depends on the length of the stems particularly the length of the peduncle (the distance from the last node to the base of the head). Some of the factors that will increase the length of the stem include: the genetic constitution of the cultivar, high fertility level especially nitrogen, low light intensity, and crop density promotes internodes elongation. An increase of 10-25% in the length of the lowest three internodes has been attributed to high nitrogen level in various crops, including semi-dwarf varieties of wheat and barley (Pinthus, 1973). Thus, a tall plant that has weak stem has a greater tendency to lodge than a semi- dwarf cultivar with stiffer straw.

Effects of Lodging

Lodging index was significantly ($P < 0.01$) affected by the main effects of rate and timing of N fertilizer application and by the interaction.

Table 5: Mean square values of phenology, lodging and growth parameters of tef as influenced by N rate and timing of application



Source of variation	Degrees of freedom	Days to panicle emergence	Lodging percentage	Plant height	Number of effective tillers
Replication	2	6.0222ns	12.183ns	14.132*	4.067ns
Rate	2	5.9556*	148.292**	67.385**	94.867**
Timing	4	8.1333**	85.318**	15.600*	21.278*
Rate*Timing	8	6.4833**	34.639**	11.256*	18.478*
Error	28	0.9508	5.716	4.222	4.329

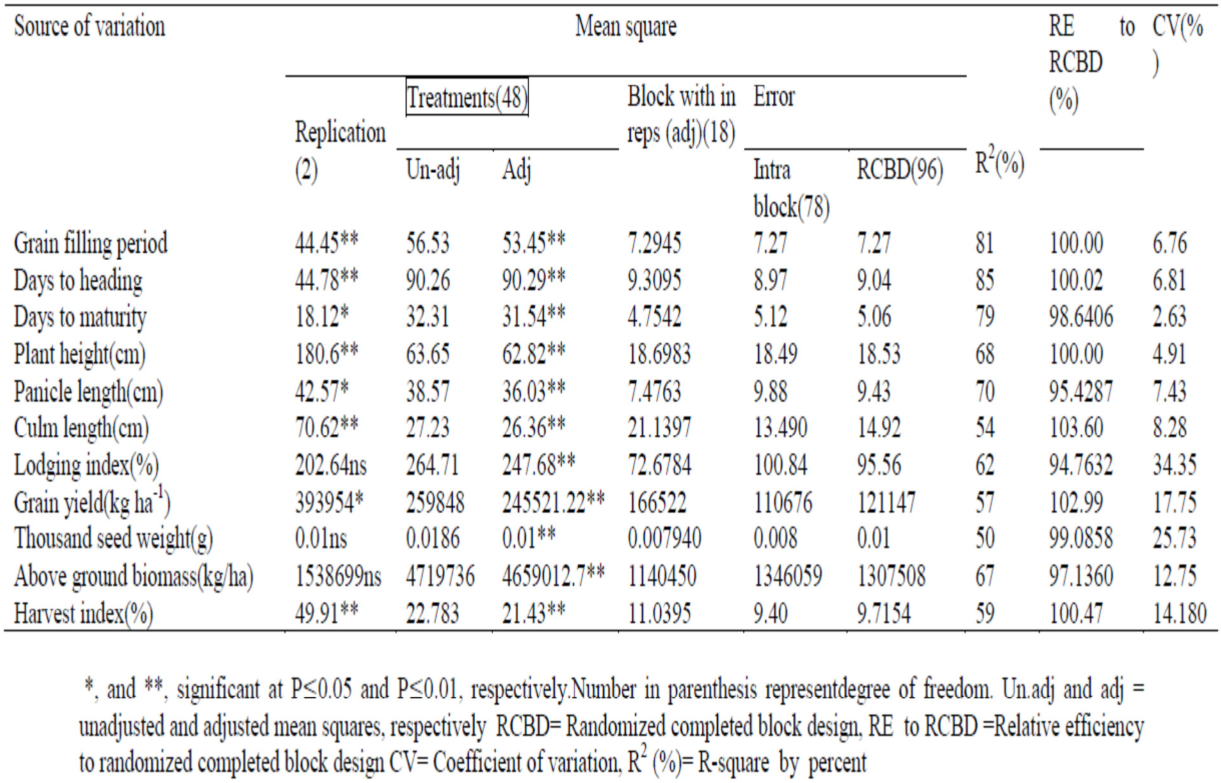
Where, ** = highly significant; * = significant; ns = Non-significant

(Source: Abraha Arefaine, 2013)

Past studies on Genetic and phenotypic variation of Tef

The variability of a crop under study is better assessed from genotypic (GCV) and phenotypic (PCV) coefficients of variations of which high GCV is breeders usually focus for the traits of interest (Solomon *et al*., 2013). Existence of high GCV among the lines indicates the possibility of selecting for some most important traits such as grain yield, panicle length, and harvest index (Kebebew et al. 2001). Heritability also the other genetic parameter to be considered since it indicates how much of the phenotypic variability has a genetic origin that gives objective information for the genetic selection process (Nechifor *et al.,* 2011). Heritability estimates need to be considered together with genetic advance, which is more important than heritability alone to predict the resulting effect of selecting the best individuals. It had been generally believed that the higher the heritability estimates of given traits, the simpler the selection procedure and the better would be the response to selection (Baloch, 2004). Determination of the interrelationships between various agronomic characters and their direct and indirect effect on grain yield also provide good information necessary for breeders in improving the productivity of crops.

Table 6*: Mean squares from analysis of variances for 12 traits of 49 tef genotypes tested at Maysiye in 2015*

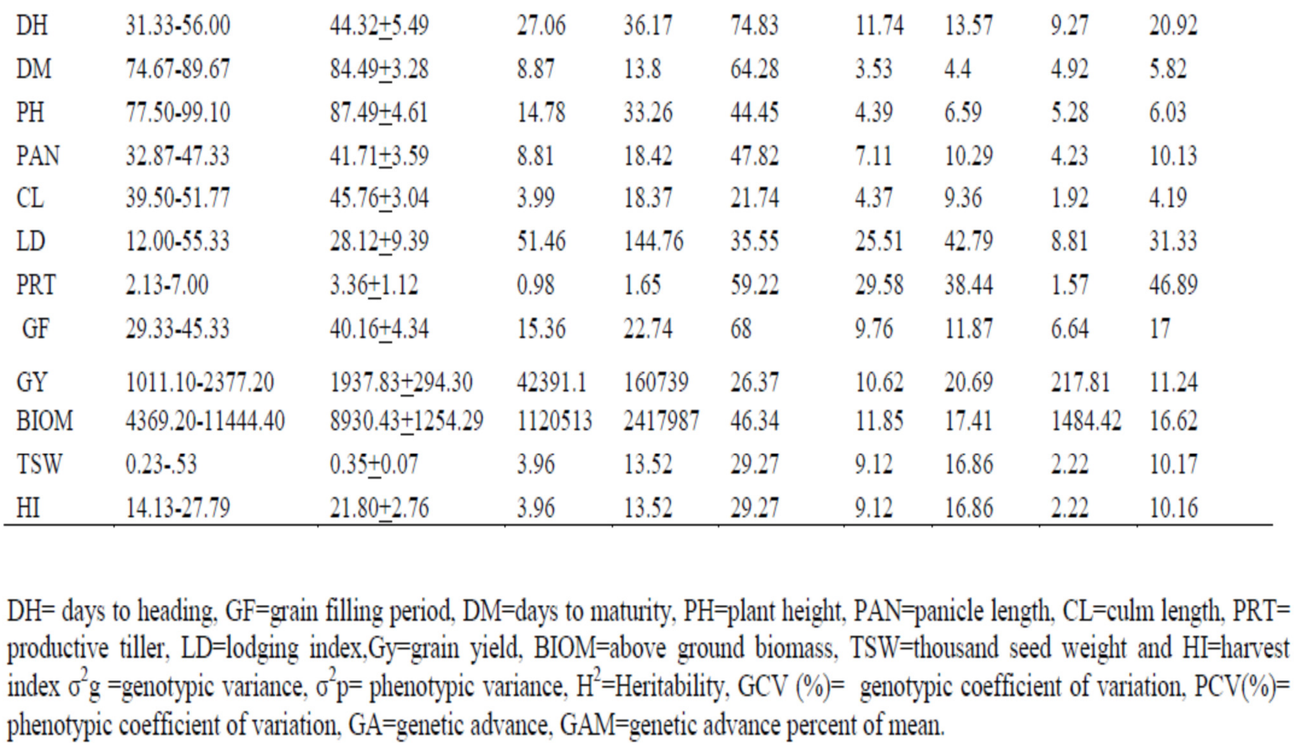


Source of variation	Mean square							RE to RCBD (%)	CV(%)
	Replication (2)	Treatments(48)		Block with in reps (adj)(18)	Error		R^2(%)		
		Un-adj	Adj		Intra block(78)	RCBD(96)			
Grain filling period	44.45**	56.53	53.45**	7.2945	7.27	7.27	81	100.00	6.76
Days to heading	44.78**	90.26	90.29**	9.3095	8.97	9.04	85	100.02	6.81
Days to maturity	18.12*	32.31	31.54**	4.7542	5.12	5.06	79	98.6406	2.63
Plant height(cm)	180.6**	63.65	62.82**	18.6983	18.49	18.53	68	100.00	4.91
Panicle length(cm)	42.57*	38.57	36.03**	7.4763	9.88	9.43	70	95.4287	7.43
Culm length(cm)	70.62**	27.23	26.36**	21.1397	13.490	14.92	54	103.60	8.28
Lodging index(%)	202.64ns	264.71	247.68**	72.6784	100.84	95.56	62	94.7632	34.35
Grain yield(kg ha^{-1})	393954*	259848	245521.22**	166522	110676	121147	57	102.99	17.75
Thousand seed weight(g)	0.01ns	0.0186	0.01**	0.007940	0.008	0.01	50	99.0858	25.73
Above ground biomass(kg/ha)	1538699ns	4719736	4659012.7**	1140450	1346059	1307508	67	97.1360	12.75
Harvest index(%)	49.91**	22.783	21.43**	11.0395	9.40	9.7154	59	100.47	14.180

*, and **, significant at P≤0.05 and P≤0.01, respectively.Number in parenthesis representdegree of freedom. Un.adj and adj = unadjusted and adjusted mean squares, respectively RCBD= Randomized completed block design, RE to RCBD =Relative efficiency to randomized completed block design CV= Coefficient of variation, R^2 (%)= R-square by percent

(Source: Chekole, *et al*., 2016)

Table 7*: The range, mean, standard deviation, estimates of variance components, broad sense heritability, and genetic advance for 12 traits of 49 tef genotypes at Maysiye in 2015*



Traits	Range	Mean ±SD	σ^2g	σ^2ph	H^2	GCV (%)	PCV (%)	GA	GAM (5%)
DH	31.33-56.00	44.32±5.49	27.06	36.17	74.83	11.74	13.57	9.27	20.92
DM	74.67-89.67	84.49±3.28	8.87	13.8	64.28	3.53	4.4	4.92	5.82
PH	77.50-99.10	87.49±4.61	14.78	33.26	44.45	4.39	6.59	5.28	6.03
PAN	32.87-47.33	41.71±3.59	8.81	18.42	47.82	7.11	10.29	4.23	10.13
CL	39.50-51.77	45.76±3.04	3.99	18.37	21.74	4.37	9.36	1.92	4.19
LD	12.00-55.33	28.12±9.39	51.46	144.76	35.55	25.51	42.79	8.81	31.33
PRT	2.13-7.00	3.36±1.12	0.98	1.65	59.22	29.58	38.44	1.57	46.89
GF	29.33-45.33	40.16±4.34	15.36	22.74	68	9.76	11.87	6.64	17
GY	1011.10-2377.20	1937.83±294.30	42391.1	160739	26.37	10.62	20.69	217.81	11.24
BIOM	4369.20-11444.40	8930.43±1254.29	1120513	2417987	46.34	11.85	17.41	1484.42	16.62
TSW	0.23-.53	0.35±0.07	3.96	13.52	29.27	9.12	16.86	2.22	10.17
HI	14.13-27.79	21.80±2.76	3.96	13.52	29.27	9.12	16.86	2.22	10.16

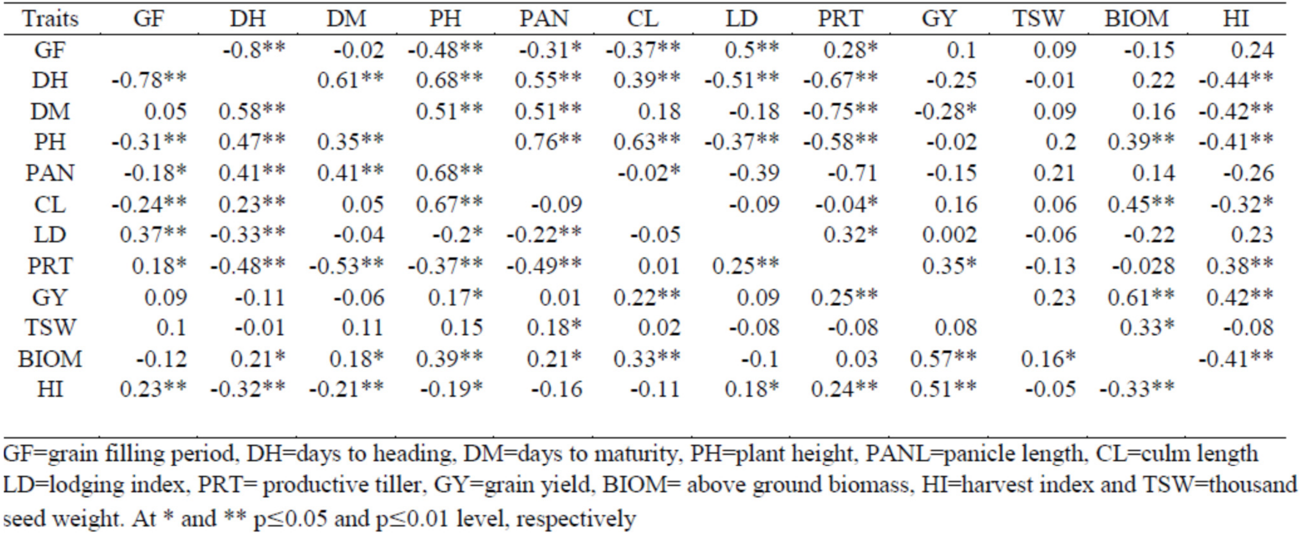
DH= days to heading, GF=grain filling period, DM=days to maturity, PH=plant height, PAN=panicle length, CL=culm length, PRT= productive tiller, LD=lodging index,Gy=grain yield, BIOM=above ground biomass, TSW=thousand seed weight and HI=harvest index σ^2g =genotypic variance, σ^2p= phenotypic variance, H^2=Heritability, GCV (%)= genotypic coefficient of variation, PCV(%)= phenotypic coefficient of variation, GA=genetic advance, GAM=genetic advance percent of mean.

(Source: Chekole , et al., 2016)

Correlation Coefficient of Lodging among others Traits

At genotypic level, lodging index had positive and significant (P<0.01) correlation with grain filling period (rg=0.5), productive tillers (rg= 0.32) and also at phenotypic level, with productive tillers (rp=0.25), grain filling period (rp=0.37) and harvest index (rp=0.18). The positive association between lodging index and grain filling period indicated that the shorter the time to grain filling, might help to reduce lodging of tef as well as the longer grain filling period giving higher grain and causes higher lodging. However, lodging index does not have correlation with grain yield. Similarly, high harvest index means high grain yield and high grain yield in turn is correlated negatively with lodging index. Varieties, which have large number of tillers, also had large number of plant population per unit area and weak stem because of competition. Consequently, the tef plants lodge easily. Therefore, selection of genotypes with short days to grain filling and maximum number of productive tillers reduce the lodging index and increase lodging index, respectively. This study was in agreement with Habte *et al*. (2015) finding that showed lodging index had negative correlation with days to head , days to maturity , plant height and culm length.

Table 8: *Genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficients for 12 grain yield and yield related traits in 49 tef genotypes*



Traits	GF	DH	DM	PH	PAN	CL	LD	PRT	GY	TSW	BIOM	HI
GF		-0.8**	-0.02	-0.48**	-0.31*	-0.37**	0.5**	0.28*	0.1	0.09	-0.15	0.24
DH	-0.78**		0.61**	0.68**	0.55**	0.39**	-0.51**	-0.67**	-0.25	-0.01	0.22	-0.44**
DM	0.05	0.58**		0.51**	0.51**	0.18	-0.18	-0.75**	-0.28*	0.09	0.16	-0.42**
PH	-0.31**	0.47**	0.35**		0.76**	0.63**	-0.37**	-0.58**	-0.02	0.2	0.39**	-0.41**
PAN	-0.18*	0.41**	0.41**	0.68**		-0.02*	-0.39	-0.71	-0.15	0.21	0.14	-0.26
CL	-0.24**	0.23**	0.05	0.67**	-0.09		-0.09	-0.04*	0.16	0.06	0.45**	-0.32*
LD	0.37**	-0.33**	-0.04	-0.2*	-0.22**	-0.05		0.32*	0.002	-0.06	-0.22	0.23
PRT	0.18*	-0.48**	-0.53**	-0.37**	-0.49**	0.01	0.25**		0.35*	-0.13	-0.028	0.38**
GY	0.09	-0.11	-0.06	0.17*	0.01	0.22**	0.09	0.25**		0.23	0.61**	0.42**
TSW	0.1	-0.01	0.11	0.15	0.18*	0.02	-0.08	-0.08	0.08		0.33*	-0.08
BIOM	-0.12	0.21*	0.18*	0.39**	0.21*	0.33**	-0.1	0.03	0.57**	0.16*		-0.41**
HI	0.23**	-0.32**	-0.21**	-0.19*	-0.16	-0.11	0.18*	0.24**	0.51**	-0.05	-0.33**	

GF=grain filling period, DH=days to heading, DM=days to maturity, PH=plant height, PANL=panicle length, CL=culm length LD=lodging index, PRT= productive tiller, GY=grain yield, BIOM= above ground biomass, HI=harvest index and TSW=thousand seed weight. At * and ** p≤0.05 and p≤0.01 level, respectively

(Source: Chekole et al., 2016)

Semi dwarf variety development and Green revolution

Major yield improvements in rice and wheat were achieved in the 1960s through intensive breeding, known as the ‘Green Revolution’. One important trait of these improved varieties was their semi-dwarf phenotype, which resulted in increased standing ability and resource reallocation into grain rather than shoot biomass (Evenson and Gollin, 2003). Plant specific hormones such as gibberellic acid (GA), brassinosteroid (BR), and auxin, as well as their signalling pathways are known to regulate plant height (Wang and Li, 2008). Likewise, microtubules have also been proven to control this trait (Sunohara *et al*., 2009).

Mutations in GA biosynthesis genes lead to lower internal concentrations of GA, resulting in dwarf phenotypes in both monocots and dicots. The altered plants are often impaired in internode elongation, having dark green leaves and shorter hypocotyls, but these phenotypes can be rescued by application of exogenous GA (Itoh *et al*., 2004; Rieu *et al*., 2008)

A novel tef mutant named *kegne*, which has a semi-dwarf phenotype, resulting in increased lodging tolerance, is characterized. Besides their short stature, *kegne* plants show right-handed twisting in young leaves and dark-grown coleoptiles as well as reduced cell sizes. Microtubule drug assays and microtubule labelling with anti-tubulin antibody indicated that the mutant phenotype is likely to be caused by a mutation in an *α-Tubulin* gene and prevailing left-handed microtubule arrays in epidermal cells. A point mutation was identified in the *α-Tubulin 1* gene which co-segregates with the *kegne* phenotype and serves as a marker for marker-assisted breeding.

Experiment

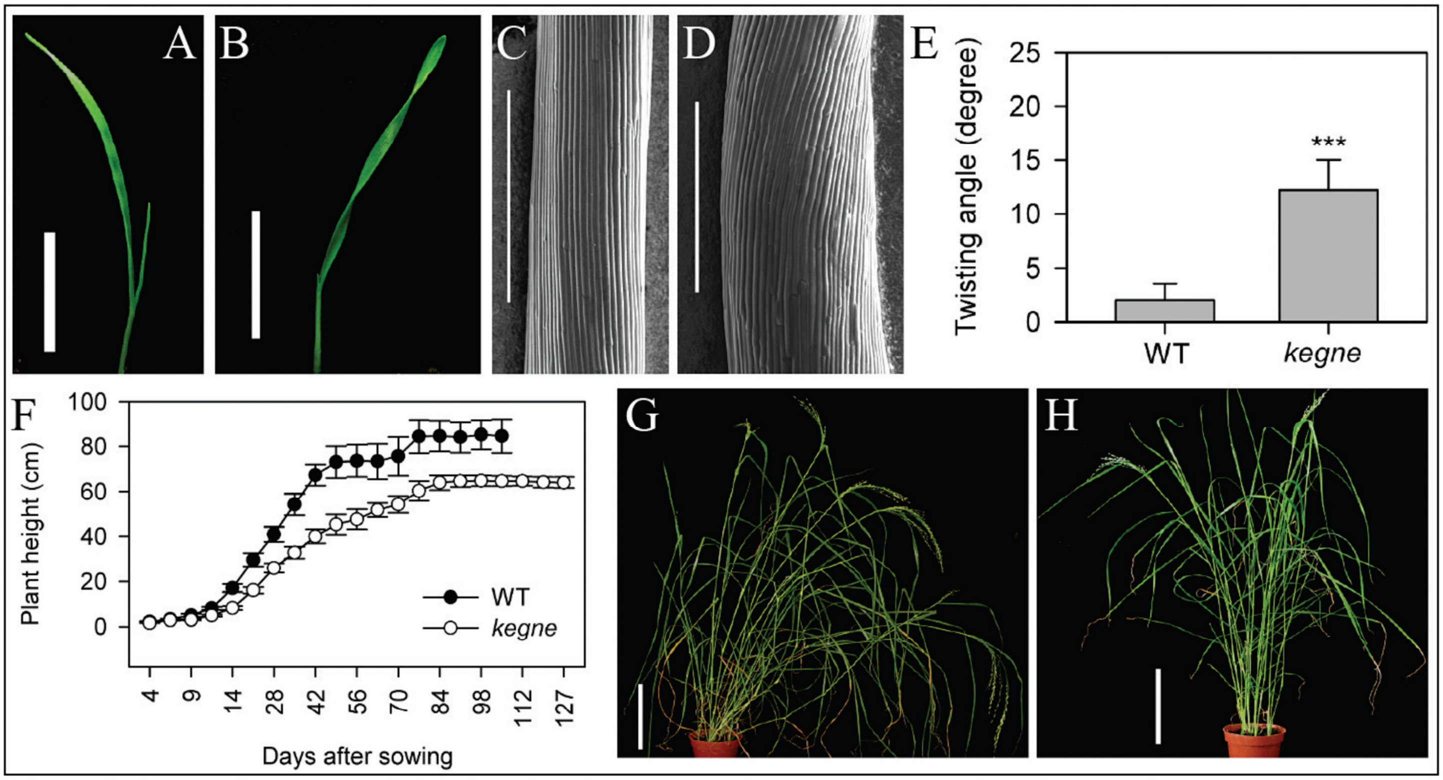
➔Plant material and growth conditions

The *kegne* mutant was derived from an EMS (ethyl methanesulphonate)-mutagenized population of the tef cultivar DZ-Cr-37 [*Tsedey*, hereafter called the wild type (WT)], generated for the ‘Tef Improvement Project’ at the University of Bern. The EMS concentration used for the mutagenesis of the tef seeds was 0.2% (v/v).

The F2 progeny of crosses between *kegne* and three improved varieties, namely *Magna* (cross name, MK), *Quncho* (QK), and *Tsedey* (TK), as well as the ecotype *Kay muri* (KK) were investigated.

For plants grown on soil, a mix of 50% topsoil, 40% peat, and 10% sand was used. The plants were fertilized with compound fertilizer (N: P: K, 2.7:1:4.3 and trace elements). Growth conditions were either short day (8 h light at 22 °C and 16 h dark at 20 °C), equal day (12 h light at 24 °C and 12 h dark at 18 °C), or long day conditions (16 h light at 22 °C and 8 h dark at 20 °C) with 65–75% relative humidity at all light regimes.

For experiments under *in vitro* conditions, seeds were surface sterilized for 5 min using 1% (v/v) sodium hypochlorite solution plus a drop of Tween-20 as wetting agent. Following sterilization, seeds were rinsed 3–4 times with distilled water and sown on square plates with half-strength Murashige and Skoog (1/2 MS) salts, 1% (w/v) sucrose, and 1% agar. The indicated amounts of hormones were added to the autoclaved and cooled media (50–60 °C). Control plates received DMSO or ethanol in the same amounts as those with treatments. Plants were grown vertically for the indicated number of days in dark or light, in a growth chamber with 14 h of light at 25 °C and 10 h of dark at 21 °C. For height- or length-related measurements, plates were scanned and the pictures were analyzed using the software ImageJ (NIH, USA). For the oryzalin experiments, plants were transferred after 10 d from oryzalin-containing media to 1/2 MS media, containing no oryzalin, and grown for a further 6 d. Plant height was measured after 10 d and 16 d. figure 2 and Table 9.

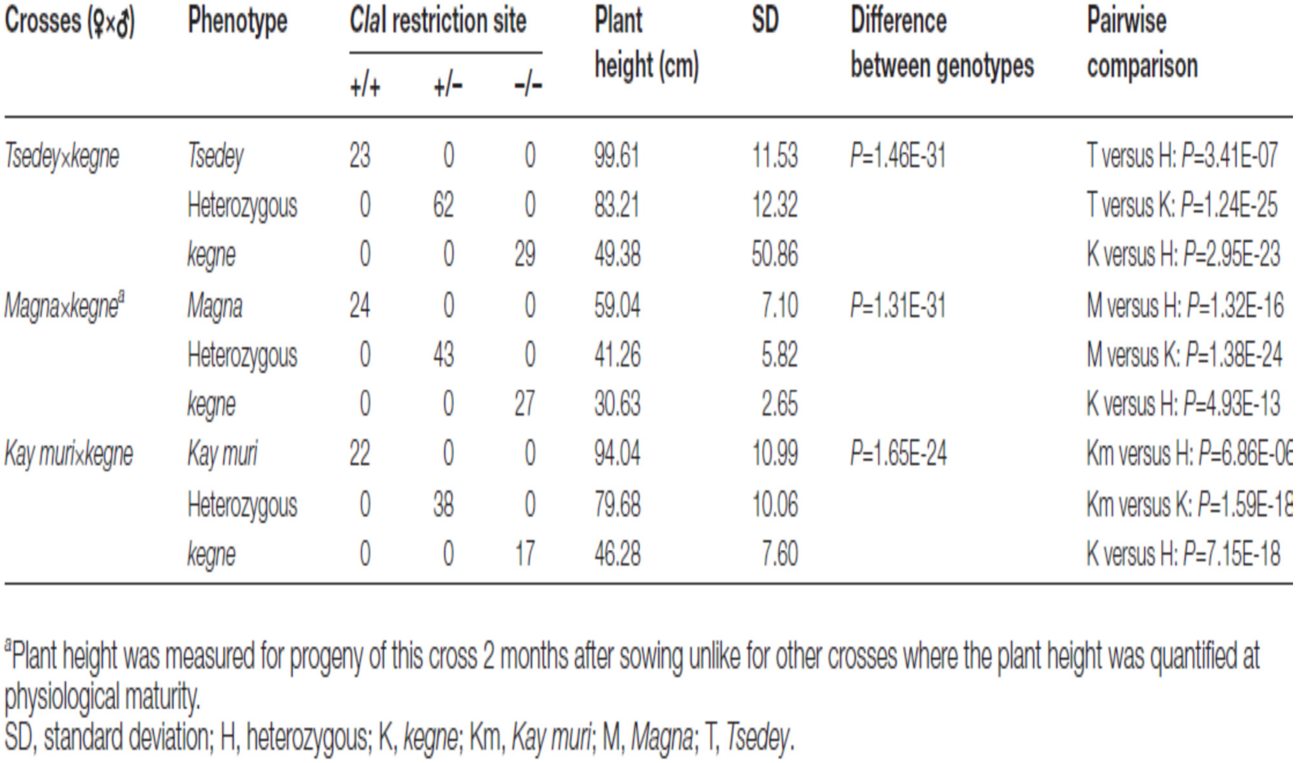


(Source: Moritz J◌st *et al* .2014.)

Figure 2: *Kegne plants twist in the right-hand direction, and are semi-dwarf and lodging tolerant.*

Nine-day-old shoots of (A) WT and (B) kegne, and 3-dayold coleoptiles of (C) WT and (D) kegne in a growth room. (E) The angle of tilting was significantly higher for kegne compared with the WT (P=1.83 E-76 after ANOVA, n=96, error bars indicate 1 SD). (F) The height of both genotypes from germination to harvest maturity; error bars indicate 1 SD (n=10). Two-month-old (G) WT and (H) kegne plants in the greenhouse. Scale bars in A and B=1 cm, in C and D=500 µM, in G and H=10 cm.

Table 9*: The co-segregation test for the three properties of kegne in F2 populations involving three crosses.*
All three properties of kegne, namely plant height, helical phenotype, and ClaI restriction (given as numbers of plants), were linked. Statistical difference and pair wise comparison was tested with ANOVA.



Crosses (♀×♂)	Phenotype	*Cla*I restriction site			Plant height (cm)	SD	Difference between genotypes	Pairwise comparison
		+/+	+/-	-/-				
Tsedey×*kegne*	*Tsedey*	23	0	0	99.61	11.53	*P*=1.46E-31	T versus H: *P*=3.41E-07
	Heterozygous	0	62	0	83.21	12.32		T versus K: *P*=1.24E-25
	kegne	0	0	29	49.38	50.86		K versus H: *P*=2.95E-23
Magna×*kegne*[a]	*Magna*	24	0	0	59.04	7.10	*P*=1.31E-31	M versus H: *P*=1.32E-16
	Heterozygous	0	43	0	41.26	5.82		M versus K: *P*=1.38E-24
	kegne	0	0	27	30.63	2.65		K versus H: *P*=4.93E-13
Kay muri×*kegne*	*Kay muri*	22	0	0	94.04	10.99	*P*=1.65E-24	Km versus H: *P*=6.86E-06
	Heterozygous	0	38	0	79.68	10.06		Km versus K: *P*=1.59E-18
	kegne	0	0	17	46.28	7.60		K versus H: *P*=7.15E-18

[a] Plant height was measured for progeny of this cross 2 months after sowing unlike for other crosses where the plant height was quantified at physiological maturity.
SD, standard deviation; H, heterozygous; K, *kegne*; Km, *Kay muri*; M, *Magna*; T, *Tsedey*.

(Source: Moritz Jöst *et'al* .2014.)

The Lodging Problem in Tef: The Way Forward

Until recent years, the tef breeding programme in Ethiopia has relied chiefly upon the indigenous resources and efforts. Notwithstanding the achievements and progress made to-date, however, lodging has yet persisted as the major constraint in tef husbandry both through direct and indirect effects. A tangible solution to the problem of lodging in tef would therefore, boost production of both grain and straw through a reduction of the lodging-inflicted losses and use of high input husbandry. It would also result in improved quality of both grains and straw for feed and would open the way for commercialization (mechanization) of tef production and eases conventional manual harvesting operations.

Combating the problem of lodging has been a sustaining major objective of the overall national tef improvement programme. But the attempts to develop lodging resistant tef varieties have encountered little success. The lack of variation for lodging resistance and other lodging resistance related traits may be a result of unfavorable associations/correlations of lodging tolerance with productivity promoting traits such as plant height, panicle length, panicle form, grain and shoot biomass yield, number of panicle branches, number of spikelet and florets, grain size and number of kernels per panicle. These associations may be caused by close correlation between culm thickness and plant height for the dwarf types in the germplasm. Short-height recombinants or segregants resulting from hybridization often have very thin stems that succumb to severe lodging as well as the short panicles that are associated with reduced grain yield.

The results from Yu et al. (2007) demonstrated that the lodging index showed positive and highly significant (P = 0.001) correlations with panicle seed weight, 100-seed weight, grain yield and shoot biomass but negative correlations with peduncle length, thus, the high yielding lines tended to lodge. Five lodging index QTLs were reported in the Kaye Murri · E. pilosa (30–5) population, and alleles that were correlated with higher lodging were from the tall, high yielding progeny with Kaye Murri contributing the majority of the lodging alleles. Two lodging QTLs showed a positive correlation between lodging and yield and other important yield related traits. This indicates that the improvement of lodging resistance in tef will be a challenging issue for breeders.

Additionally, culm length, number of internodes, and second inter node length were positively correlated with lodging, while crown diameter showed a negative correlation, as it would be expected. An interesting marker that co-localized with a lodging QTL was one of the lignin biosynthesis gene markers, PAL (Phenylalanine ammonia lyase from rice, X16099) controlling stem cellulose. Because of limited success in combating the problem of lodging in tef, we are taking a multi-dimensional approach involving the use of genetic (breeding) methods, cultural practices, anti-lodging plant growth regulators (agronomic) and machinery as illustrated in Fig.3. Considering the genetic approach to combating lodging, various methods including direct selection, hybridization, induced mutation techniques and genomics will hopefully provide a synergistic effect. Several genetic studies have found linkage between lodging and plant height. This has been used for indirect selection for barley, rice, wheat and maize. Similarly, in tef, short plant stature shows less lodging compared to a tall plant. Therefore, the development of a dwarf tef plant is an alternative approach which could result in a plant that is resistant to lodging while responding to fertilizer application so that the seed yield per unit area is increased. Currently, Targeting Induced Local Lesions IN Genomes (TILLING) has been implemented in tef in order to obtain dwarf tef plants and to identify genetic variation at the sequence level of dwarf genes in tef. Approximately, 6000 families mutagenized by ethyl methanesulfonate (EMS) were screened for semi-dwarf phenotypes and about 45 candidates were obtained (Zerihun Tadele at University of Bern, unpublished data). In addition, a modified method known as Eco tilling was also applied on large number of 500 non mutagenized accessions to detect useful genetic variations in natural populations. So far, two candidate genes of interest from tef have been cloned. The first gene is a tef homolog of DWARF4 (Sakamoto *et'al*., 2006) a mutation that resulted in a semi-dwarf rice plant from a TILLING population. The second gene is a tef homolog of the rice gene, High Tillering and Dwarf 1 (HTD1; Zou *et al*., 2006) generated in an Ecotilling population. Additional TILLING and Ecotilling populations are under characterization for candidate gene functions using orthologous candidate genes considered to affect plant height. This information would be useful to improve the tef lodging syndrome in terms of (1) conventional breeding using mutant lines; (2) marker assisted breeding using sequence variations; and (3) transformational approach of delivering semi-dwarf genes into the tef plant.

Our optimism is based on the belief that (1) tef is a cereal and the problem has long been combated in the other cereal crops; (2) the future holds scientific promise; and (3) the future generations of tef breeders will be equipped with better knowledge and skills.

i) **Lodging Syndrome**:

A multi-faceted approach involving different disciplines has been suggested to combat the problem of lodging in tef (Figure 3). This involves: a) cultural practices such as row planting, optimum plant population density (low seed rate), optimum nitrogen fertilization, and deep seeding; b) use of chemicals that reduce the height of the plant especially of the length of the culm and thereby indirectly increase the tolerance of the plant to lodging; c) use of improved farm implements and machinery for planting; and d) breeding for lodging tolerant cultivars. The latter, in turn, involves: i) direct selection for dwarf and semi-dwarf plant height, stem strength and large seed size; b) intra- and inter-specific hybridization with a view to increase the number of crosses so as to increase the chances for crossing over to get recombinants with breakage of the apparent linkage between plant height and stem thickness; c) induced mutagenesis to get mutants with lodging resistance traits; and d) modern molecular or genomics approach including TILLING and Eco-TILLING, QTL analysis, comparative and association mapping, in vitro culture techniques, and genetic transformation.

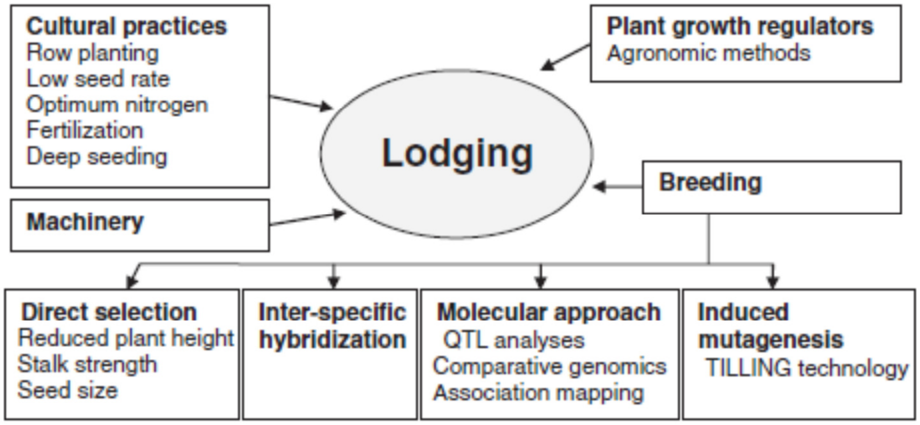


Figure 3*: Schematic diagram showing envisaged integrated methods for combating lodging in tef.*

SUMMARY AND CONCLUSION

A major cause of low productivity of tef is lodging, the permanent displacement of the stem from the upright position. Tef has a tall and slender stem which is susceptible to lodging caused by wind and rain.

In addition, when fertilizer is applied to increase yield, stems of tef grow taller and become even more susceptible to lodging, resulting in significantly reduced quantity and quality of grain and straw. Moreover, lodging makes harvesting by hand difficult and mechanical harvesting nearly impossible. The average yield reduction due to lodging is estimated at 17%.

The presence of considerable variations among tef genotypes has been observed indicating the potential of improving the crop through selection of genotypes that combine the traits of interest. It also suggested the country has rich genetic resources for the crop as center of origin and diversity. Days to maturity, biomass and productive tillers had medium to high values of heritability whereas, except days to maturity failed at low genetic advance as percent of mean biomass, productive tillers and harvest index had medium to high values of genetic advance indicating that these traits were less influenced by environmental factors. Considering all genetic variability components biomass yield and harvest index can be used for, direct selection while, early maturity, and productive tillers indirect selection of genotypes to improve tef grain yield in areas where terminal drought is the major constraints of production. Tef genotypes that exhibited short days to heading and grain filling period need to be considered as potential genetic materials for improving tef production through selection. It can be concluded that the importance of continuing the study of variability in tef genotypes at different location to identify which traits can be used for causal selection of genotypes for grain yield.

A multi-faceted approach involving different disciplines has been suggested to combat the problem of lodging in tef (Fig. 3). This involves: a) cultural practices such as row planting, optimum plant population density (low seed rate), optimum nitrogen fertilization, and deep seeding; b) use of chemicals that reduce the height of the plant especially of the length of the culm and thereby indirectly increase the tolerance of the plant to lodging; c) use of improved farm implements and machinery for planting; and d) breeding for lodging tolerant cultivars. The latter, in turn, involves: i) direct selection for dwarf and semi-dwarf plant height, stem strength and large seed size; b) intra- and inter-specific hybridization with a view to increase the number of crosses so as to increase the chances for crossing over to get recombinants with breakage of the apparent linkage between plant height and stem thickness; c) induced mutagenesis to get mutants with lodging resistance traits; and d) modern molecular or genomics approach are the ways to combat lodging problems in Tef production.

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