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Screening of ethyl methane sulphonate mutagenized tef [*Eragrostis tef* (Zucc.) Trotter] population identifies Al-tolerant lines

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ABSTRACT

About 15,000 M_2 seeds of ethyl-methane-sulphonate (EMS)-mutagenized population were screened along with Al-tolerant and sensitive checks and the M_0 variety. Strongly acidic soil with an external application of a toxic Al-solution and exposure to moisture stress was used to maximize selection pressure. Twenty-one M_2 plants with root lengths of greater than the mean of the tolerant check were selected and planted for seed production. Candidate M_3 plants were investigated for Al-tolerance and for morpho-agronomic traits under greenhouse and field conditions, respectively. Highly significant differences were observed for Al-tolerance between the candidate mutant lines and the M_0 ($P < .001$), and between mutant lines and the sensitive check ($P < .001$). Similarly, significant differences were observed between the mutant lines for 16 of the 20 quantitative traits measured. This study is the first to report successful induction of enhanced Al-tolerance in tef by using EMS mutagenized population.

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KEYWORDS
Aluminum; ethyl methane sulphonate (EMS); mutation breeding; tef

1. Introduction

The global population is projected to reach 9 billion by the year 2050. The world will need 70–100% more food to feed this population (FAO 2009). This in turn requires mean annual increment of 44 million metric tons per year for the coming years. Maximizing productivity of crops through development of high-yielding crop varieties in potential growing environments is one of the strategic options available to meet the global food demand. Improving the current low yields of marginal growing environments such as acid soils is also another approach to lift up the global agricultural produce (FAO 2009; Godfray et al. 2010; Tester & Langridge 2010).

Tef [*Eragrostis tef* (Zucc.) Trotter] ($2n = 4x = 40$) is the most widely produced and consumed cereal crop in Ethiopia. In terms of area of cultivation, it is the leading cereal crop followed by maize and wheat. According to the Central Statistical Authority (CSA, 2015), the area covered by tef during the 2014/2015 cropping season was over 3 million hectares or 30% of the total area occupied by cereals in the country. As a gluten-free cereal, tef is currently gaining popularity worldwide (Spaenij-Dekking et al. 2005). Besides, tef is also grown as a pasture crop in several countries (Assefa et al. 2011).

Aluminium toxicity and other acidity-related soil fertility problems are among the major constraints affecting tef production in Ethiopia (Dubale 2001; IFPRI 2010).The problem is widespread in the high rainfall areas of the north western, western, southern, and south western parts of the country (Schlede 1989; Abebe 2007). These areas have good agricultural potential to offset poor productivity of areas under recurrent drought. Worldwide, development of varieties tolerant of acid soils has been a sound alternative to liming, and other non-genetic management options in the production of globally important crops (Rao et al. 1993; Hede et al. 2001).

Mutation breeding has been used to induce variability and develop improved varieties of various crop species (Jain 2005; Mba 2013). In tef, mutation breeding was started in 1972 using gamma radiation, with the primary objective of inducing lodging-resistant phenotypes that lacked in the natural population (Tefera et al. 2001). Since recent past, a chemical mutagen, ethyl methane sulphonate (EMS), has been successfully utilized to induce semi-dwarf tef variants resistant to lodging (Esfeld et al. 2009; Jöst et al. 2015). Several studies reported that EMS produces a large number of (genome-wide) non-lethal point mutations in plants (Greene et al. 2003; Till et al. 2003, 2004).

Despite widespread problems of soil acidity and Al-toxicity affecting tef production, breeding for tolerance to Al-toxicity in tef has not been a research focus in Ethiopia. The aim of this study was to isolate and characterize Al-tolerant lines from an EMS-induced M_2 population of tef.

2. Materials and methods

2.1. Induction of mutation

Seeds of an improved tef variety, *Tsedey* (DZ-Cr-37), that is, M_0, were mutagenized in the Tef Improvement Project at the Institute of Plant Sciences, University of Bern in Switzerland, using 0.2% of EMS for 8 hours. About 10,000 plants from the first generation after mutagenesis (M_1 population) were self-pollinated and about 7000 non-chimeric M_2 families were obtained. M_2 seeds pooled from 5000 M_2 families were used for selection on acid soil along with M_0 variety, *Tsedey* (DZ-Cr-37) and an Al-tolerant local selection.

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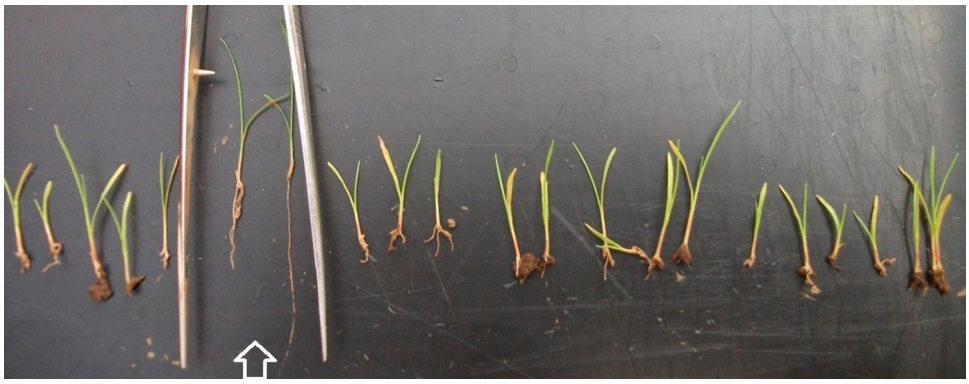

Figure 1. Early root pruning effects of Al-toxicity and nutrient deficiency symptoms in sensitive M_2 mutant lines (tolerant selections are marked with forceps).

2.2. Selection of Al-tolerant mutants

Strongly acidic soil with pH (H_2O) 1:2.5 of 4.5 was collected from the major acid-soil-affected district, Banja, in the north western Ethiopia. The soil was irrigated with 222 µM $AlK(SO_4)_2.12H_2O$ until a pH (H_2O)1:2.5 of 4.0 was achieved (Islam et al. 2004). Fifteen thousand M_2 seeds were planted in pots (10 cm diameter) in a greenhouse of the Amhara Agricultural Research Institute, at Bahir Dar, Ethiopia. A local landrace with Al-tolerance, the M_0 variety, and a sensitive check were also planted for comparison. The plants were fertilized with NPK at the rate of 100, 109, and 137 µg g^{-1} of soil, respectively, using NH_4NO_3 and KH_2PO_4. The pots were uniformly watered with 222 µM $AlK(SO_4)_2.12H_2O$ (pH 4) for the first 2 weeks (14 days). Elimination of seedlings with poor root development (poorly anchored) was started one week after planting, using fine-tipped forceps (Figure 1).

The overall activities conducted in selection and characterization of the mutant lines are presented in Figure 2.

The concentration of $AlK(SO_4)_2.12H_2O$ was doubled to 444 µM after the second week in order to increase selection pressure. This concentration further differentiated the seedlings and enabled further elimination during the third week.

Since Al-toxicity impedes root development of sensitive plants, it enhances the vulnerability of such plants to drought of even a short duration (Little 1989; Foy 1992). Hence, during the fourth week, green and apparently tolerant seedlings were subjected to moisture stress by discontinuing watering for 96 hours. The Al-tolerant landrace showed wilting after the fourth day. All seedlings of the mutant population that showed temporary wilting earlier were eliminated. This procedure allowed further identification of sensitive plants with poorly developed root system. At this stage, all the seedlings of the M_0 and the sensitive check were eliminated.

Twenty-eight days after planting, the soil was washed and the roots of the Al-tolerant landrace were measured. The mean plus the standard deviation of the root length of the Al-tolerant landrace was used as truncation point to select the Al-tolerant mutant plants. All mutant plants that had root length of greater than the truncation point were transplanted into normal growing medium in pots for seed production (Table 1).

In order to exclude sensitive segregants, subsequent generations of mutant lines were advanced by subjecting the seedlings to 350 µM $AlK(SO_4)_2.12H_2O$. Single plants with the longest root were preserved per mutant line.

2.3. Experiment I: evaluation of M_3 lines for Al-tolerance

Genetic stock: Twenty-one M_3 lines with root length of above 47 mm were planted in pots (10 cm) in a greenhouse along

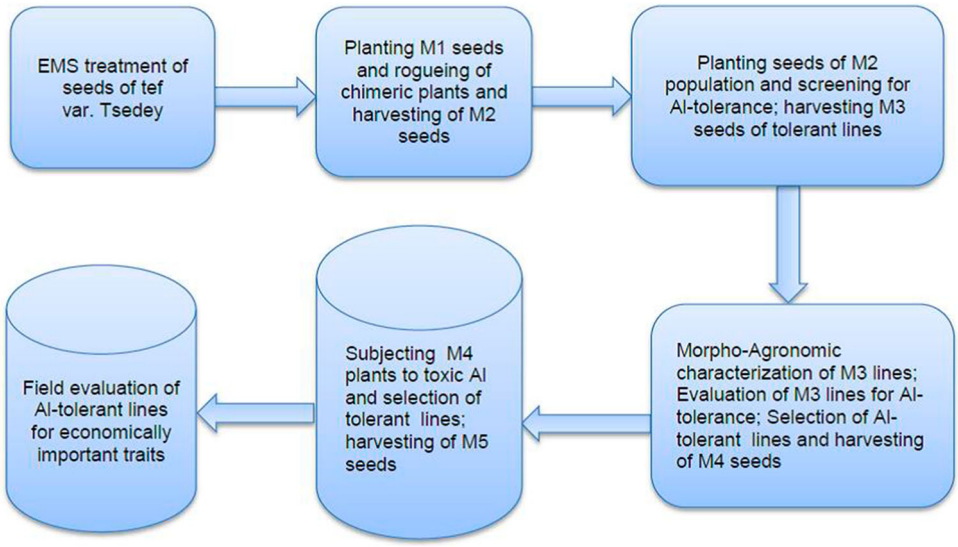

Figure 2. Schematic illustration of mutation induction, isolation, evaluation and characterization activities.

Table 1. Description of root length of mutant selections compared to the local Al-tolerant selection.

Selections	*N*	Root length (cm) Mean	Min.	Max.	Std.
Al-tolerant landrace	70	34.46	15	70	12.35
Mutant selections measured for root length	217	31.40	10	70	11.45
Mutants selected, that is, above truncation point (47 mm)	21	54.60	47	70	6.24

Notes: Truncation point = Mean + Std. of Al-tolerant landrace = 47 mm; *N*, number individual plants measured; Std, standard deviation; Min, minimum; Max, maximum.

with the M_0 variety, an Al-tolerant landrace, and a sensitive check, *Holeta Key*.

Experimental set-up: A sample of an acidic soil with a pH (H_2O) 1:2.5 of 4.45 and pH (KCl) of 3.68 was collected from the *Banja* District of north western Ethiopia. The soil was analyzed for various physico-chemical properties at the Amhara Design and Supervision Works Enterprise, Soil Chemistry and Water Quality Section, Bahir Dar, Ethiopia (Table 2).

In order to compute tolerance indices, the experiment was established under limed and unlimed conditions for all the test genotypes. Accordingly, the acid soil was limed to a pH of 6.2 by applying 8.5 g of $CaCO_3$ (99.5%) powder per kilogram of dry soil (equivalent to 17 ton $CaCO_3$ or lime ha^{-1}) (Nyachiro & Briggs 1988) and incubating the limed soil for seven days in a greenhouse. Before planting, the soil was fertilized with NPK as indicated above. Seeds were planted in 10 cm diameter pots. The experiment was set up in a randomized complete blocks design, with three replications under limed and unlimed condition.

Data collection: Shoot and root length (mm) data were collected from each pot 28 days after planting from randomly selected plants, and the mean of seven plants was used for statistical analysis. Root and shoot dry weights (mg) were recorded on the basis of 10 randomly selected plants per replication after oven drying at 65°C for 72 hours.

Tolerance indices (relative values) were computed as the ratio of the measured parameters under unlimed *versus* limed conditions.

Statistical analysis: Measurements of each parameter under unlimed soil and tolerance indices were subjected to analysis of variance, and means separation using GenStat Statistical Software Version:17.10013780 (GenStat 2014).

2.4. Experiment II: morpho-agronomic characterization of mutant lines

Experimental set-up: The twenty-one M_3 lines, along with M_0 variety, were grown at the Adet Agricultural Research Centre, north western Ethiopia, during the 2014 cropping season under natural field conditions. A randomized complete block design with two replications was used with a plot size of 0.6 m^2 and inter-row spacing of 20 cm. The seeds were drilled in the row with a seed rate of 15 kg ha^{-1}. At tillering, the plants within each row were thinned to an intra-row spacing of 5 cm. Fertilizers were applied with rates of 59.8 kg ha^{-1} P_2O_5 and 23.4 kg ha^{-1} N at planting, and 16.6 kg ha^{-1} N at tillering.

Data collection: Days to 50% panicle emergence and days to 75% maturity were recorded on a plot basis. Culm length; number of internodes; first basal internode length (cm); first basal internode diameter (mm); second basal internode length (cm); second basal internode diameter (mm); panicle length (cm); number of panicle branches; number of spikelet; number of florets per spikelet; grain yield/panicle (g); and phytomass (g) were recorded on the basis of the main shoots of seven randomly selected plants from the central row. Counts of spikelet per panicle and the number of florets per spikelet were made for the basal, middle, and apical parts of the main shoot panicle.

Number of fertile tillers/plant, grain yield/plant (g), phytomass yield/plant (g), and the harvest index (%) were recorded on the basis of seven randomly selected plants from the central row. Culm and grains were dried in an oven at 70°C for 48 hours, as described by Hobbs and Sayre (2001), to determine the above-ground biomass and the harvest index. Mean values of these samples were used to describe each line for the traits under consideration.

Statistical analysis: Analysis of variance and cluster analysis were performed to assess the variability among the mutant lines and estimate the relatedness among the lines using GenStat Statistical Software Version:17.10013780 (GenStat 2014).

3. Results

3.1. Variability for Al-tolerance

Analysis of variance revealed the presence of highly significant differences between the mutant lines for both the tolerance indices and actual measurements under unlimed conditions (Table 3). Orthogonal contrast between the Original cultivar (M_0) and the mutant lines also showed highly significant differences for all the parameters. However, the mutant lines and the Al-tolerant landrace did not show significant differences for all the parameters. Figure 3 also showed equivalent shoot and root growth of the tolerant check and the mutant lines. The significant difference observed between the sensitive check and the M_0 showed that the M_0 was less sensitive to Al-toxicity than the sensitive check.

Table 4 shows the responses of the mutant lines in terms of tolerance indices and actual root and shoot growth under unlimed conditions, along with their rank. Relative root dry weight (RRDW) and relative root length (RRL) were effective in the investigation of the tolerance to Al-toxicity because they indicate the relative performance of the genotype under unlimed conditions and limed conditions. Values over 100% indicate that the genotype performed well under unlimed conditions relative to limed condition. Compared to RRL, RRDW gives a better measure of tolerance because it takes into account the root density. Accordingly, except

Table 2. pH and other physico-chemical properties of the soil used for the pot experiment.

	pH (H_2O) 1:2.5	pH (KCl)	Exchangeable bases (Cmol(+) kg^{-1}) Ca	Mg	Na	K	Cation exchange capacity (CEC) (Cmol(+) kg^{-1})	*N* total (%)	Available Phosphorus (mg kg^{-1})	Exchangeable Acidity (Cmol(+) kg^{-1})	Exchangeable Al
Limed	6.23	5.48	46.75	0.05	0.01	0.61	22.00	0.478	5.75	5.68	0
Unlimed	4.45	3.68	13.03	0.12	0.12	0.56	23.40	0.384	5.33	18.64	4.16

Table 3. Analysis of variance for Al-tolerance parameters among mutant lines.

Source of variation	d.f.		RRL (%)	RRDW (%)	RL	RDW
Block	2					
TRT	23	*P* value	<.001	<.001	<.001	<.001
		F-static	14.26	13.99	4.97	7.07
Original cultivar (M_0) vs. ML	1	*P* value	<.001	<.001	0.002	<.001
		F-static	36.61	27.53	11.19	18.46
Local vs. ML	1	*P* value	0.059	0.299	0.309	0.34
		F-static	3.74	1.1	1.06	0.93
Sensitive check vs. ML	1	*P* value	<.001	<.001	<.001	<.001
		F-static	102.8	103.5	30.55	79.98
Original cultivar (M_0) vs. Local		*P* value	<.001	<.001	0.003	0.020
		F-static	33.39	20.76	10.02	5.81
Original cultivar (M_0) vs. Sensitive check	1	*P* value	0.005	<.001	0.121	0.002
		F-static	8.76	12.71	2.49	11.31
Residual	46					
Total	71					

Note: ML, mutant lines; RRL, relative root length; RRDW, relative root dry weight; RL, root length unlimed; RDW, root dry weight unlimed.

for ML99, all the mutant lines were superior to the Original cultivar, M_0 and the sensitive check. This result was expected because the selection was severe and only 0.14% plants from the original 15,000 seeds were retained.

3.2. Variability for morpho-agronomic traits

Analysis of variance indicated significant differences for 16 of the 20 morpho-agronomic traits analyzed (Table 5). The mutant lines did not show significant difference for hundred seed weight, number of internodes, first basal internode diameter, and second basal internode diameter. Minimum and maximum values of each trait are presented along with the mean of all the genotypes and the original cultivar, M_0 (Table 5).

Agronomically and economically important traits like days to 50% panicle emergence, days to maturity, seed and biomass yield per main shoot and whole plant, panicle length, and number of panicle branches all showed considerable variation around the mean of the original cultivar, M_0, suggesting that the mutagenesis and the selection procedures employed have resulted in variability both in positive and negative directions. The maximum whole plant seed yield and whole plant biomass yields of 26.78 and 58.5 g were obtained for the selection ML139, with a gain of 58.0% and 55.1% over the mean of the original cultivar, M_0 for both traits, respectively. Similarly, a maximum harvest index of 54.57% was recorded for the selection ML61 with a gain of 22% over the M_0.

No difference was observed for most of the qualitative traits between the M_0 and the mutant lines. But some mutant lines like ML153 were distinct enough in developing an extremely loose panicle form compared to the M_0 and most of the mutant lines (data not shown). Hierarchical cluster analysis using the Euclidean distance between groups showed that the relatedness among the lines was very close with a maximum dissimilarity value of less than 0.1 for most of the mutant lines (Figure 4). The original cultivar, M_0 did not show distinct clustering from the mutant lines and was most closely related to the line ML209.

4. Discussion

This study has resulted in the successful isolation of Al-tolerant lines that exceeded the M_0 in all of the tolerance parameters and actual growth measurements under unlimed conditions. Most of the mutant lines were better or equivalent to the Al-tolerant landrace grown in strongly acidic Acrisols of north western Ethiopia. This suggests that the EMS application has successfully induced variability for Al-tolerance in the original tef population. The screening techniques employed in this study, that is, combined use of strongly acidic soil along with application of Al in the form of AlK $(SO_4)_2.12H_2O$, and subjecting seedlings to severe drought were efficient at identifying Al-tolerant lines. This study is the first to report the use of EMS for induction of genetic variability for Al-tolerance in tef. Induction of mutation has been used to increase genetic variability for Al-tolerance in other plants. For instance, Nawrot et al. (2001) have reported increased level of Al-tolerance in barley after mutagenic treatment of four varieties with *N*-methyl-*N*-nitroso urea and sodium azide. Similarly, treatment of Al-sensitive Arabidopsis with EMS resulted in variants that grew in highly toxic Al condition (Kelly et al. 2006).

As an orphan crop, genetic control and physiological mechanisms of Al-toxicity tolerance in tef has not been studied. In other globally important cereals such as wheat, rice, maize, sorghum barley, genetic control and tolerance mechanisms have been well studied. In wheat, barley, and sorghum, few major genes that control activation of transmembrane channel and exudation of organic acids upon exposure of roots to toxic Al concentration determine Al-toxicity tolerance. In tolerant varieties, these organic acids complex the toxic Al (Al^{3+}) and prevent it from attaching to the negatively charged sites of the cell wall and plasma membrane (Delhaize et al. 1993; Kochian et al. 2005; Magalhaes et al. 2007; Wang et al. 2007; Ryan et al. 2009). In rice and

Figure 3. Contrasts between root and shoot growth of the tolerant check, the Original cultivar (M_0) and selected mutant tef lines under unlimed, acid soil conditions.

Table 4. Means and ranks of mutant lines measured in terms of tolerance indices and actual growth under unlimed condition.

	RRDW (%)		RRL(%)		RL (mg)		RDW (UL)	
Mutant lines	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
ML-209	142.04	1	152.70	2	80.05	3	35.67	1
ML-149	133.85	2	121.10	14	56.9	18	28.50	11
ML-153	119.01	3	137.40	5	84.33	1	32.67	2
ML-96	115.24	4	130.50	10	65	14	31.00	5
ML-207	114.80	5	152.00	3	72.24	8	32.00	3
ML-48	111.52	6	127.20	12	77.95	4	31.83	4
Dabo Banja	106.13	7	137.30	6	72.43	7	26.50	16
ML-183	104.59	8	155.60	1	76.71	5	29.33	7
ML-205	102.40	9	136.80	7	68.33	11	29.00	8
ML-184	100.84	10	151.70	4	82.38	2	25.17	18
ML-22	100.14	11	127.30	11	71.67	9	28.33	12
ML-133	98.04	12	133.40	9	68.62	10	28.50	10
ML-117	97.34	13	118.50	15	52.67	20	27.50	15
ML-98	96.94	14	134.90	8	74.14	6	28.33	13
ML-61	93.91	15	110.60	17	66.62	12	29.83	6
ML-94	91.90	16	82.90	21	51.33	21	28.83	9
ML-194	88.74	17	114.30	16	65.33	13	25.00	19
ML-139	87.14	18	84.80	20	54.14	19	24.83	20
ML-148	82.39	19	89.50	18	58.43	16	24.33	21
ML-49	75.25	20	123.30	13	64.05	15	27.83	14
ML-173	68.10	21	85.90	19	57.9	17	26.50	17
T*sedey*	65.41	22	71.00	23	47.58	23	20.17	22
ML-99	61.98	23	76.00	22	49.43	22	20.00	23
Holeta Key	33.55	24	37.00	24	35.19	24	11.33	24
Mean	95.5		116.30		64.7		27.21	
LSD (5%)	17.99		23.11		15.8		5.287	
CV (%)	11.5		12.10		14.8		11.8	

Notes: RRDW, relative root dry weight; RRL, relative root length; RL, root length; RDW, root dry weight.

maize, exclusion and internal detoxification mechanisms controlled by several quantitative genes are involved in Al-toxicity tolerance (Maron et al. 2008, 2010; Huang et al. 2009, 2012; Yamaji et al. 2009; Xia et al. 2010, 2011; Yokosho et al. 2011; Chen et al. 2012; Guimaraes et al. 2014). Comparative mapping study among important cereals crops indicated extensive synteny or colinearity of Al-tolerance loci among genomes of rice, wheat, barley, rye, oat, maize, and sorghum (Jardim, 2007). This suggests that similar mechanisms could operate in tef tolerance to Al-toxicty.

The significant difference between the mutant lines for most of the agronomic traits showed that EMS has successfully induced variations in most of the traits measured. This suggests that many genes controlling these traits were affected by the EMS treatment. Earlier studies have reported that EMS produced a large number of point mutations in different plants' species (Greene et al. 2003; Till et al. 2003, 2004).

Despite considerable level of variation observed for most of the traits measured in this study, the level of variation was narrower than the ones observed in natural populations

Table 5. Minimum, maximum and mean values and significance tests of the selected mutant lines of tef for 20 morpho-agronomic traits.

		Minimum		Maximum					
No	Trait	Value	ML	Value	ML	Mean ± (SE)	M_0	*F*-value	*P* value
1	Days to maturity	98.00	ML207	106.00	ML49 ML99	101.20 ± (1.68)	99.00	6.45	<.001
2	Days to 50% panicle Emergence	49.00	ML98	57.50	ML49 ML61 ML139 ML153 ML183	54.50 ± (1.51)	55.00	4.91	<.001
3	Number of fertile tillers	3.67	ML173	10.86	ML139	6.32 ± (1.22)	5.50	3.69	0.002
4	Main shoot biomass (g)	3.30	ML98	10.86	ML22	6.39 ± (1.28)	5.65	3.88	0.002
5	Main shoot seed weight (g)	2.50	ML194	6.61	ML61	4.65 ± (0.89)	5.22	2.51	0.02
6	Whole pant Biomass (g)	24.35	ML48	58.51	ML139	35.78 ± (3.16)	37.73	13.54	<.001
7	Whole plant seed weight(g)	8.98	ML183	26.78	ML139	14.64 ± (2.42)	16.93	6.99	<.001
8	Hundred seed weight (mg)	27.00	ML96 ML194	31.00	M_0	28.66 ± (1.20)	31.00	1.63	0.135
9	Harvest Index	26.55	ML183	54.57	ML61	40.72 ± (5.60)	44.80	3.05	0.007
10	Plant height (cm)	74.36	ML98	96.14	ML148	84.11 ± (2.34)	77.32	14.23	<.001
11	Culm length(cm)	43.57	ML207	57.50	ML173	48.69 ± (2.32)	46.43	3.68	0.002
12	Panicle length	31.43	ML184	42.93	ML61	37.19 ± (1.99)	34.93	6.94	<.001
13	Number of internodes	2.71	ML117 ML133	3.79	ML139	3.19 ± (0.54)	3.50	0.61	0.87
14	First basal internode length (cm)	3.00	ML149	5.06	ML133	3.90 ± (0.32)	3.46	5.42	<.001
15	First basal internode diameter (mm)	1.54	ML98	2.15	ML22	1.88 ± (0.19)	1.96	1.39	0.228
16	Second basal internode length (cm)	6.64	ML96	9.43	ML61	8.04 ± (0.68)	7.43	2.45	0.023
17	Second basal internode diameter mm)	1.60	ML149	2.11	ML61	1.89 ± (0.18)	2.02	1.51	0.177
18	Number of panicle branches	21.79	ML98	31.71	ML153	26.08 ± (2.53)	25.50	2.06	0.05
19	Mean number of florets	5.01	ML61	7.06	ML48	6.03 ± (0.58)	5.68	2.39	0.026
20	Number of spikelets per panicle	17.12	ML207	26.81	ML61	21.17 ± (1.89)	20.60	2.74	0.013

Note: ML, mutant lines; Original cultivar (M_0) is the variety *Tsedey*; *F*, *F*-statistic or variance ratio; SE, standard error.

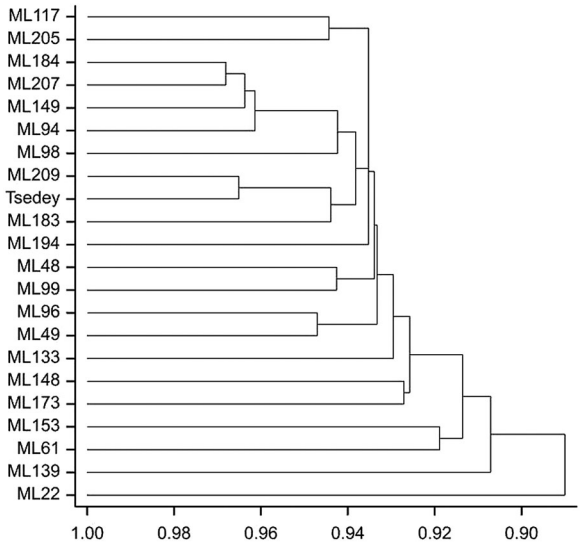

Figure 4. Dendrogram showing similiarity among the mutant lines based on 20 morpho-agronomic traits.

for traits such as first basal internodes diameter, second basal internodes diameter, first and second internodes length, total culm length, and number of internodes (Assefa et al. 1999, 2000, 2001). On the other hand, the value of agronomically important traits such as seed yield per plant, shoot biomass per plant, and harvest index were higher in the present study than those reported by the above authors. This was expected because the mutation treatment was made on the background of agronomically superior variety. This finding suggests the possibility of enhancing tolerance to Al-toxicity of Al-sensitive but popular tef cultivars through EMS-induced mutation and subsequent rigorous screening.

5. Conclusion

This study documented the successful induction of mutations for Al-tolerance and several morpho-agronomic traits by using EMS. The screening procedures were efficient in identifying Al-tolerant lines. Induction of mutation by EMS may be utilized to develop Al-tolerant varieties without sacrificing important agronomic traits, especially when used on the genetic background of popular varieties. The combined screening techniques utilized in this study can be easily used in screening of different crop species for tolerance to Al-toxicity in developing countries where high-tech solution cultures cannot be used.

Quantitative variations observed among the mutant lines in these study suggest possible inolvement of several genes in tef Al-toxicty tolerance. Nevertheless, systematic investigation is imperative to determine genetic control and tolerance mechanism to Al-toxicty tolerance in tef. Further, development of mapping population and molecular markers is also a prerequisit to identify genes involved in Al-toxicity tolerance.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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