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Quantitative responses of tef [*Eragrostis tef* (Zucc.) Trotter] and weeping love grass [*Eragrostis curvula* (Schrad.) Nees] varieties to acid soil

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Abstract

Tef [*Eragrostis tef* (Zucc.) Trotter] is the most widely produced and consumed cereal crop in Ethiopia. It is a gluten-free crop with growing popularity worldwide. Unlike most globally important cereals, tef has not yet been bred for tolerance to soil acidity and to aluminium toxicity. This experiment was conducted to assess the quantitative responses among some grain and pasture varieties of tef. Strongly acidic soil (pH 3.94 and acid saturation of 78%) was used to evaluate the tef varieties. A highly Al-tolerant weeping love grass [*Eragrostis curvula* (Schrad.) Nees] variety, Ermelo, was used as a check. A randomized complete block design (RCBD) with 4 replications was used to evaluate the materials under limed and unlimed conditions. Measurements were taken on different root and shoot parameters. The result indicated the presence of genetic variability among the tef varieties for root length, shoot length, root dry weight and shoot dry weight. All the tef varieties were inferior to the variety Ermelo of *E. curvula* in their Al tolerance. The brown seeded tef varieties consistently showed better Al-tolerance than the white seeded ones. A similar pattern was also observed for tolerance indices, which were computed as ratio of each parameter under unlimed versus limed condition. Highly significant correlations (r>0.9) were observed for all the parameters used to assess Al-tolerance in this experiment. This first systematic work demonstrated the presence of genetic variability for tolerance to soil acidity and Al-toxicity within tef varieties. This variability suggests the possibility to launch strategic breeding of the crop with specific adaptation to acid soil prone areas.

Keywords: aluminium toxicity, Eragrostis tef, genetic variability, screening, soil acidity.

Abbreviations: ARL-Average root length; ASHL-Average shoot length; RDW-Root dry weight; RSHDW-Relative shoot dry weight; RSHL-relative shoot length; RRDW-Relative root dry weight; RRL-Relative root length; RTI-Root tolerance index; SHDW-Shoot dry weight, SH:RT-shoot to root ratio.

Introduction

Acid soils (soils with pH < 5.5 in the surface layer) constitute 3,950 million ha or 30% of the world's total ice-free land. In Africa, 22% or 659 million ha of the total 3.01 billion ha land has acid soils (von Uexk"ull and Mutert, 1995; Malcolm and Andrew, 2003). The main problems of crop production on mineral toxicities related to dissolved acid soils are aluminium, manganese, and iron, and deficiencies of phosphorus, calcium, magnesium, and molybdenum (von Uexk"ull and Mutert, 1995; Hede et al., 2001; Kochian et al., 2004). Sixty-seven percent of the acid soils of the world have Al-toxicity problem (Eswaran et al., 1997). In Ethiopia, acidity-related soil fertility problems are major production constraints, reducing productivity of the major crops grown in the country (Paulos, 2001; IFPRI, 2010). About 41% of the total land area has acid soils and 33% of this area has Altoxicity (Schlede, 1989). Abebe (2007) noted that the soil acidity problem of Ethiopia is mainly related to some of the Alfisols, and most Oxisols and Ultisols soil classes that occur in the west, north-western, south-western and southern parts of the country. Tef [Eragrostis tef (Zucc.) Trotter] is the most widely produced and consumed gluten-free cereal crop in Ethiopia (Spaenij-Dekking et al., 2005; CSA., 2010). Annually, it occupies about 2.5 million hectares or 28% of the total area covered by cereals in the country (CSA., 2010). Consequently, it is among the worst affected crops by soil

acidity. Beyond Ethiopia, countries such as Eritrea, USA, the Netherlands and Israel produce small areas of tef as a grain crop (Spaenij-Dekking et al., 2005). On the other hand South Africa, India, Pakistan, Australia, Uganda, Kenya and Mozambique grow tef as a forage or pasture crop (Assefa et al., 2010). Tef responds poorly to fertilizer application on acid soils (Mamo and Killham, 1987; Mamo et al., 1996). The use of lime, compost, manure and other organic fertilizer sources are recommended to cope with problem of soil acidity. However, these options are constrained by several factors. In the tropics, most acid soils have a strong buffering capacity against amendments of lime (Rao et al., 1993). Hence, large amount of lime is needed to normalize the pH. Most resource-poor farmers in the tropics are constrained by unavailability, transport, and the high cost of the large volumes of lime needed to treat the soils (Rao et al., 1993; von Uexk"ull and Mutert, 1995). In addition, lime has low mobility and its mechanical incorporation in to the subsoil is also often difficult for small-scale farmers without tractors and subsoil rippers. Consequently, when surface soils are amended with lime, it fails to increase the pH of the sub-soil, resulting in restricted root growth and, therefore, poor plant growth (Rao et al., 1993; von Uexk"ull and Mutert, 1995; Abebe, 2007). Limited root growth also increases the vulnerability of plants to drought of even short duration (Foy,

1992). This is particularly important because many acid soils have inherently low water holding capacity (Little, 1989; Haynes and Mokolobate, 2001). The use of organic matter in the form of manure and compost can make a significant contribution to reduce soil acidity (Wong and Swift, 2003). Nevertheless, in countries like Ethiopia, where animal manure and crop residues have widespread use as fuel and animal feed, respectively, large-scale use of this option is not common (Schlede, 1989; IFPRI, 2010). The problem of soil acidity on cultivated land is further aggravated by the use of acid-forming chemical fertilizers. In particular, the predominant inorganic fertilizers used in Ethiopia are urea and diammonium phosphate (DAP) (Abebe, 2007). Both these fertilisers increase soil acidity when converted to nitrate nitrogen by releasing hydrogen ions (Barak et al., 1997; Abebe, 2007). Worldwide, development of varieties tolerant to aluminium has been a sound alternative to liming, and other non-genetic management options in the production of globally important crops such as wheat, rice, maize, barley, sorghum and rye (Foy and Murray, 1998; Pinto-Carnide and Guedes-Pinto, 1999; Hede et al., 2001; Paterniani and Furlani, 2002; Kochian et al., 2005; Portaluppi et al., 2010). On tef, no systematic study has been made on tolerance to Al-toxicity. However, a closely related forage species, weeping love grass [Eragrostis curvula (Schrad.) Nees] is known to have a high level of tolerance to soil acidity (pH 4.1 with high level of exchangeable Al) (Foy et al., 1987; Miles and Villiers, 1989). This species is considered to be one of progenitors of tef (Ketema, 1993). This research was conducted in order to investigate the quantitative responses among some grain and pasture varieties of tef.

Results

Genetic variability under unlimed treatments

Under unlimed conditions the acid soil (pH [KCI] 3.94 and acid saturation of 78%) caused variety specific responses for root length, shoot length, root dry weight, and shoot dry weight. The analysis of variance revealed highly significant differences between the varieties (p<0.01). The single degree of freedom contrast showed a highly significant (p<0.01) difference between E. curvula variety Ermelo and the E. tef varieties for all the parameters measured (Table 1). Contrast analysis between the pasture and food grain tef varieties, however, did not show significant differences for the parameters. On the other hand, comparison between brown seeded and white seeded tef varieties showed a highly significant difference (P<0.01) for all the parameters measured (Table 1). Stunted shoot growth coupled with a severe root pruning effect were observed in the more sensitive varieties, which is typical effect of Al-toxicity in unlimed soils (Figs 1 and 2).

For all the parameters measured, the variety Ermelo of *E. curvula* showed better growth under unlimed conditions and was followed by the brown seeded tef varieties Dima, Emmerson and SA Brown, in that order. Among the tef varieties, the highest and lowest values for average root length and average shoot length were recorded for the varieties Dima and Witkop, respectively. Similarly, substantial variability was observed among the varieties for root dry weight and shoot dry weight (Table 2). Among the tef varieties, the lowest and highest root dry weights recorded were 3.38mg and 10.45mg for Highveld and Dima, respectively. For shoot dry weight Quncho and Highveld gave the smallest weights of 7.38mg and the brown seeded tef variety, Dima, gave the highest weight of 20.9 mg.

Variability for tolerance indices (relative values) and shoot to root ratio

Highly significant differences were observed for tolerance indices of all the growth parameters that were measured as a ratio of the value under unlimed versus limed conditions. These indices indicated the extent of stress created by the unlimed soil relative to the limed soils. Single degree of freedom contrast between E. curvula and E .tef varieties showed a highly significant difference and contributed for the larger proportion of the variation between the varieties for all the tolerance indices. Highly significant differences were also observed between the brown and white seeded varieties of tef. However, no significant differences were observed between pasture and grain varieties of tef for all the growth parameters (Table 3). The tolerance indices for variety Ermelo of E. curvula ranged between 0.81 for root length to 0.98 for root dry weight. This reflects extremely high tolerance of the E. curvula variety to Al-toxicity and other stresses associated with the highly acidic soils. Lime had negligible effects on all the growth parameters measured for E. curvula. This result is consistent with earlier research reported for the species (Foy et al., 1987; Miles and Villiers, 1989; Foy and Murray, 1998). The tolerance indices or relative values among tef varieties ranged between 0.13-0.39mm for relative root length; 0.23-0.43mm for shoot length; 0.12-0.36mg for root dry weight and 0.11-0.31mg for shoot dry weight, indicating substantial variability between the tef varieties. Within tef varieties, the brown seeded grain variety Dima consistently gave the highest tolerance indices for all the growth parameters measured. The tef varieties generally responded more strongly to liming than E. curvula. However, severe suppression of growth of tef varieties under unlimed conditions resulted in very low tolerance indices of tef for all the parameters (Table 4). Shoot to root ratio for the limed treatments gave highly significant differences and the values ranged between 2.7 for SA Brown to 1.95 for Yilmana. Under unlimed condition significant differences were not observed for shoot to root ratios. Generally, shoot to root ratios were reduced under unlimed condition (Table 4). A product-moment correlation coefficient indicated a high (>0.9) and highly significant correlation (p<0.01) between the growth parameters (Table 5). In screening experiments for Al-tolerance, shoot and root dry matter are usually recorded to capture variability in root density that cannot be accounted for by the length parameters per se (Miles and Villiers, 1989; Liu, 2005). The high correlation between shoot and root length, and corresponding dry matter values observed in this experiment indicated the possibility that data recorded on length parameters can be used instead of root density.

Discussion

The primary effect of Al-toxicity is inhibition of root growth, which eventually results in hampered absorption of water and nutrients, and consequently stunted growth of plants (Little, 1989; Delhaize and Ryan, 1995; Hede et al., 2001; Deborah and Tesfaye, 2003; Kochian et al., 2004; Miyasaka et al., 2007). In this study, high levels of root pruning and severely stunted growth was observed among the tef varieties grown under unlimed condition. Root and shoot growth parameters of seedlings are commonly used to evaluate genetic variability and screen for acid or Al-tolerant varieties in many crop and forage species (Little, 1989; Foy and Murray, 1998; Hede et al., 2001; Liu, 2005; Dai et al., 2011).

Table 1. Analysis of variance and orthogonal contrasts for growth parameters of nine tef varieties and E. curvula variety Ermelo grown in unlimed highly acidic soil^a.

Source of variation	d.f.		ARL	ASHL	RDW	SHDW
Block	3					
Varieties	9	P value	< 0.001	< 0.001	< 0.001	< 0.001
		F statistic	13.85	9.13	11.58	19.62
E. curvula vs. E. tef	1	P value	< 0.001	< 0.001	< 0.001	< 0.001
		F statistic	83.51	38.56	67.03	120.56
Pasture vs. food grain varieties	1	P value	0.420	0.5	0.419	0.560
(E. tef)		F statistic	0.67	0.47	0.67	0.35
White vs. brown seeded	1	P value	0.001	< 0.001	0.008	< 0.001
varieties		F statistic	12.87	18.08	8.33	18.41
(E. tef)						
Residual	27					
Total	39					

^ad.f-degrees of freedom; ARL-Average root length; ASHL-Average shoot length; RDW-Root dry weight; SHDW-Shoot dry weight.



Fig 1. Shoot growth of E. tef varieties and E. curvula in limed and unlimed acid soil.

In this experiment, statistically significant correlations were observed between all the parameters indicating the appropriateness of using these parameters for similar studies on tef. Under unlimed condition, root and shoot growth of tef varieties was generally lower than that of the variety Ermelo of *E. curvula*. This is mainly attributed to the inherent high tolerance of *E. curvula* to highly acidic soils (Foy et al., 1987; Miles and Villiers, 1989).

Similarly, the maximum tolerance index recorded for E. tef (0.44) for shoot length was very low compared to values of over 0.9 recorded for E. curvula. As tolerance index is the ratio of growth under unlimed (toxic) versus limed (nontoxic) condition, the low tolerance indices of tef varieties was visible in the luxurious growth versus the severely stressed plants of tef varieties under limed and unlimed condition, respectively. The most tolerant tef variety, Dima, and the highly tolerant E. curvula variety showed equivalent growth under unlimed conditions. However, since growth of Dima under limed condition was luxurious as compared to the variety Ermelo of E. curvula, considerable difference was observed for tolerance indices of these two varieties (Table 2, Figs 1 and 2). Assefa et al. (2010) described the existing tef cultivar development strategy of Ethiopia as breeding for general adaptation. The E. tef varieties tested in this study were also not intentionally developed for acid or Altolerance. Recent figures on variety development in tef indicate a decline in genetic gain mainly because of a lack of specifically adapted varieties, and because of high genotype

by environment interactions (Assefa et al., 2010). The differential response of tef varieties to acid soil, detected in this study, also suggests the need to launch strategic breeding of the crop with specific adaptation to Al-tolerance in Ethiopia.

Material and methods

Plant materials

Four grain and 5 pasture tef (*E.tef*) varieties, along with the variety Ermelo of E. *curvula*, were evaluated under greenhouse conditions at the University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Experimental set up

Acid subsoil with a pH of (KCl) 3.94 and acid saturation of 78% was used in the experiment. The acid soil was analysed for pH and other chemical properties in the Soil Fertility Analytical Services laboratory of KwaZulu-Natal Department of Agricultural and Environmental Affairs (Table 6). The acid soil was limed to a pH of 6.21 (KCl) with application of 3.6 g of CaCO₃ (97%) powder per kilogram of dry soil and was incubated for seven days in a greenhouse. Before planting, the soil was fertilized with NPK at the rate of 100, 109 and 137 μ g.g⁻¹ of soil, respectively, using NH₄NO₃ and KH₂PO₄ as fertilizer sources. Twenty seeds of each variety

Table 2. Growth of tef	varieties grown under	unlimed highly acidic soil	$(pH_{(CD)}=3.94).$

		U		(01)		
	Seed		ARL	ASHL	RDW	SHDW
Varieties	colour	Use	(mm)	(mm)	(mg)	(mg)
Witkop	White	Pasture	15.45 a	8.85 a	4.90ab	8.50a
Quncho	White	Food	16.85 a	12.35ab	4.525ab	7.38a
Etsub	White	Food	18.60 a	12.25ab	4.50ab	8.28a
Rooiberg	Brown	Pasture	16.40 a	15.35bcd	5.30ab	10.10ab
Yilmana	White	Food	19.15 a	13.90bc	4.075a	7.85a
Highveld	Brown	Pasture	20.00 a	11.30ab	3.375a	7.38a
SA Brown	Brown	Pasture	25.35 ab	15.60bcd	5.550ab	11.35ab
Emmerson	Brown	Pasture	37.60 bc	18.50cd	7.55bc	14.80b
Dimma	Brown	Food	48.20 c	19.95de	10.45c	20.90c
E. curvula var Ermelo	Brown	Pasture	72.20 d	24.10e	14.25d	30.45d
Mean			29.0	15.21	6.45	12.70
F statistic			13.85	9.13	11.58	19.62
P value			< 0.001	< 0.001	< 0.001	< 0.001
LSD (5%)			14.47**	4.38	2.92	4.95
CV (%)			34.4	19.8	31.2	26.8

^aMeans in the same column followed by the same letter are not significantly different at p=0.05. ^bARL-Average root length; ASHL-Average shoot length; RDW-Root dry weight; SHDW-Shoot dry weight



Fig 2. Root length E. tef varieties and E. curvula var Ermelo grown in limed and unlimed acid soil.

were planted per pot (10 cm) and then thinned out to 15 plants soon after emergence. All the nine tef varieties and the variety Ermelo of *E. curvula* were planted in limed and unlimed soil, forming 20 treatment combinations. The experiment was setup in a randomized complete blocks design with 4 replications.

Data collection and analysis

Shoot and Root length (mm) data were collected from each pot 30 days after planting from randomly selected plants and the average of five plants was used for statistical analysis. Root and shoot dry weights (mg) were recorded on the basis of five 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. In addition, the shoot to root ratio was computed under both limed and unlimed conditions. Analysis of variance and single degree of freedom contrast, mean separation by Fisher's least significant difference test, and correlation coefficients, were carried out using the GenStat Statistical Software Version:14 (GenStat., 2009).

Table 3. Analysis of variance and orthogonal contrasts of tolerance indices (relative values) of growth parameters under limed and unlimed conditions^a.

Source of variation	d.f.		RRL	RSHL	RRDW	RSDW
Block	3					
Varieties	9	P value	<.001	<.001	<.001	<.001
		F statistic	20.06	46.44	19.08	37.1
E. curvula vs E.tef	1	P value	<.001	<.001	<.001	<.001
		F statistic	151.72	380.23	158.73	317.74
Pasture vs food grain varieties	1	P value	0.04	0.201	0.932	0.453
(<i>E. tef</i>)		F statistic	0.85	1.72	0.01	0.580.
White vs brown seeded varieties (E.tef)	1	P value	0.002	0.001	0.031	0.041
		F statistic	12.47	12.89	5.2	4.58
Residual	27					
Total	39					

^aRRL-Relative root length; RSHL-relative shoot length; RRDW-Relative root dry weight; RSHDW-Relative shoot dry weight.

Table 4. Tolerance indices and shoot to root ratios of tef varieties ^{a,b}

							SH:RT	SH:RT
	Seed						(DW)	(DW)
Varieties	colour	Use	RRL	RSHL	RRDW	RSHDW	Limed	unlimed
Witkop	White	Pasture	0.13a	0.21a	0.17a	0.12a	2.42abc	1.75
Quncho	White	Food	0.15ab	0.32bc	0.18ab	0.14a	2.10a	1.65
Etsub	White	Food	0.15ab	0.27ab	0.13a	0.12a	2.08a	1.86
Rooiberg	Brown	Pasture	0.16ab	0.31abc	0.22ab	0.15a	2.88	2.07
Yilmana	White	Food	0.16ab	0.31abc	0.12a	0.12a	1.95a	1.92
Highveld	Brown	Pasture	0.18ab	0.28ab	0.14a	0.12a	2.42abc	2.17
SA Brown	Brown	Pasture	0.27abc	0.33bc	0.25ab	0.20ab	2.78bc	2.02
Emmerson	Brown	Pasture	0.29bc	0.39cd	0.22ab	0.15a	2.82c	2.01
Dimma	Brown	Food	0.39c	0.44d	0.36b	0.31b	2.29abc	2.24
<i>E. curvula</i> var	Brown	Pasture	0.81d	0.93e	0.98c	0.96c	2.19ab	2.21
Ermelo								
Mean			0.27	0.38	0.28	0.239	2.39	1.99
F statistics			20.06	46.44	19.08	37.10	3.21	0.61
P value			<.001	<.001	<.001	<.001	0.009	0.778
LSD (5%)			0.1344	0.0871	0.0836	0.1240	0.5402	NS
CV (%)			34.7	15.9	42.7	35.8	15.6	25.2

^aMeans in a column followed by the same letter are not significantly different at p=0.05.

^bRRL-Relative root length; RSHL-relative shoot length; RRDW-Relative root dry weight; RSHDW-Relative shoot dry weight; SH: RT- shoot to root ratio; DW-dry weight.

Table 5. Correlation coefficients between the various growth parameters measured in the study^a.

Parameter	ARL	ASHL	RDW
ARL	-		
ASHL	0.9314**	-	
RDW	0.9287**	0.9229**	-
SHDW	0.9161**	0.9512**	0.9490**
		DDUUD 1	1 1 GITDIN GI

ARL-Average root length; ASHL-Average shoot length; RDW-Root dry weight; SHDW-Shoot dry weight

Table 6. Chemical properties of unlimed and limed sub-soil used for the stud	ly.
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	Clay	pН	N	la	K	Ca	Mg	Total		Acid	Р	Zn	Mn	Cu	Mid infrar	ed
Sample	%)	(KCl)			(mg/l)	(mg/l)	(mg/l)	Cation	Exchangeable	satura					estimate	
			mg/l	ESP (%)				(Cmol/L)	acidity (Cmol/L)	tion (%)	mg/l	mg/l	mg/l	mg/l	Organic Carbon	N (%)
Unlimed	48	3.9	1.98	0.2	109	69	17	3.5	2.74	78	1	0.8	4	1.2	<0.5	0.07
Limed	47	6.2	3.08	0.1	119	1351	83	7.77	0.04	1	1	0.6	2	0.7	<0.5	0.05

ESP-Exchangeable sodium percentage

Conclusion

The highly acid subsoil used for the experiment was effective at exposing the intraspecific genetic variation in tef for tolerance to Al-toxicity and other acidity associated stresses. The tef varieties used in this experiment were not intentionally bred for Al-tolerance and the considerable variation observed among the tef varieties suggests the possibility of selecting tef varieties with high level of Altoxicity tolerance among diverse tef accessions. In this regard, deliberate screening of tef accessions collected from acid soil areas could be an appropriate starting point. A consistent association of brown seed colour with Al-tolerance in this experiment suggests that further research might show a clear genetic linkage between brown seed colour and acid soil tolerance.

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