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Genetic variation and trait association of tef [*Eragrostis tef* (Zucc.) Trotter] evaluated under optimal and moisture stressed environments

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Abstract

Tef [*Eragrostis tef* (Zucc.) Trotter] is one of the major cereal crops grown in the Horn of Africa for food and as an export commodity for its unique nutritional qualities. Moisture stress is the leading yield limiting factor of tef production in northern Ethiopia. The objectives of this study were to assess the genetic variability present among 144 tef genotypes of varied population and to identify important agronomic traits with high heritability and correlations for effective breeding. The genotypes were evaluated using four experiments laid down in a 12 x 12 lattice design under moisture stressed and non-stressed conditions in the northern Ethiopia. Main shoot panicle seed weight had high genotypic coefficients of variation (GCV) of 22.4% and 25.9% under non-stressed and stressed conditions, respectively. Grain yield had GCV values of 17.6% and 20.0% in the corresponding environments. Heritability was highest under optimum condition than the moisture stressed, which is valuable to conduct effective selection. A path-coefficient analysis indicated that direct selection for high biomass, harvest index and late maturity could increase grain yield under optimal conditions, while under moisture stress conditions early maturity, high biomass and harvest index were important direct selection criteria for tef breeding aiming for drought tolerance.

Keywords: Correlation, Ethiopia, genetic advance, heritability, path-analysis.

Abbreviations: ADARC _Adet Agricultural Research Center; ARARC_ Areka Agricultural Research Center; BARC_ Bako Agricultural Research Center; BYLD_biomass yield; CSA_ Central statistics authority; DM_days to 75% maturity; DPE_days to panicle emergence; DZARC_ Debre-Zeit Agricultural Research Center; GAM_ genetic advance as percent of the mean; GCV _Genotypic coefficients of variance; GPF_grain filling period; GYLD_grain yield; H_ broad sense heritability; HARC_Holleta Agricultural Research Center; HI_harvest index; LODG_lodging index; MARC _Melkassa Agricultural Research Center; NPT_number of tillers per plant; PCV _phenotypic coefficients of variance; PLHT_ plant height; PNLG_ panicle length; PSW_main shoot panicle seed weight; SARC_ Sirinka Agricultural Research Center.

Introduction

Tef is the principal food crop supporting more than 50 million people in the Horn of Africa. Further, it is increasingly used in other parts of the world for gluten-free food products (Assefa et al., 2011). In Ethiopia, tef is the most widely grown crop and its production area has expanded from 1.99 million ha in 2004 (CSA, 2004) to 3.02 million ha in 2015 (CSA, 2015), a 51% increase. Correspondingly, total grain production of tef has increased from 1.67 million tons to 47 million tons, translating a productivity of 0.84 to 1.58 t ha^{-1} (CSA, 2015). In Ethiopia tef yields are significantly low when compared to maize, sorghum and wheat yields of 3.43, 2.37 and 2.54 t ha^{-1}, respectively (CSA, 2015). Low yields of tef are attributed to its susceptibility to lodging, moisture stress, seed shattering, and poor pre- and post-harvest agronomic systems (Assefa et al., 2011). Tef varieties with high grain yield, but high tolerance of moisture stress and lodging are the major goals of plant breeders. This could be achieved through exploitation of the genetic variability present in tef germplasm, especially using landraces for their unique traits.

The progress of selection in crop improvement programs depends on the extent and magnitude of genetic variation (Aniol, 2001). Previous genetic variability studies (Assefa et al., 1999, 2000, 2001a; Admas and Belay, 2011; Ayalew et al., 2011; Shiferaw et al., 2012) reported a wide variability in yield and yield components in tef germplasm in Ethiopia.

The magnitude of heritability and the correlation of traits determine genetic advancement through direct or indirect selection (Falconer and Mackay, 1996; Sleper and Poehlman, 2006). This is particularly important when the heritability of traits is low for effective selection (Singh and Chaudhary, 1977; Dabholkar, 1992). Previous studies (Admas and Belay, 2011; Assefa et al., 2001a; Ayalew et al., 2011; Chanyalew, 2010; Debebe et al., 2013) indicated that heritability, genetic advance and correlation of traits of tef genotypes are variable depending up on the test populations and test environments. Therefore, identification of highly heritable and correlated traits in the targeted environment is important for maximum selection response in tef breeding. Path analysis is one of the powerful statistical tools that permit the measurement of the direct influence of one variable upon another. It helps to identify the most influential predictor variable useful for simultaneous selection (Singh and Chaudhary, 1977; Dabholkar, 1992). Previous path analysis studies in tef reported that harvest index and biomass yield had strong direct effects on grain yield, indicating the importance of these traits for selection for high grain yield under optimal conditions (Ayalew et al., 2011; Ayalneh et al., 2012; Debebe et al. 2013).

A recently released tef variety, namely DZ-Cr-387 (Quncho), has been promoted nationally for its farmers preferred traits such as high grain yield, white seed color, high biomass yield and good ‘Enjera’ making quality. However, this variety has poor performance and was poorly adopted by tef growers in moisture stressed environments in Ethiopia. Targeted breeding of tef for drought prone environments is needed in Ethiopia. Furthermore, grain yield and quality traits such as white seed colour and good *Enjera* qualities are important attributes for tef breeding. Therefore, the objectives of this study were to assess the genetic variability present among a diverse tef population and to identify important agronomic traits with high heritability and correlations for effective breeding in moisture stressed environments.

Results and discussion

Genotypes mean performance

Genotypes under the three non-stressed conditions showed highly significant ($p \leq 0.01$) variations for all traits evaluated (Abraha et al., 2016). Test environments and genotype by environment interactions also showed highly significant differences ($p \leq 0.01$) for all traits except the lodging index. Similarly, in the one stressed environment, highly significant ($p \leq 0.01$) differences were observed between genotypes for all traits except for biomass yield (Abraha et al., 2016). Significant difference of genotypes for yield and yield components were previously reported in tef (Assefa et al., 2000, 2001a; Adnew et al., 2005; Chanyalew, 2010; Admas and Belay, 2011; Shiferaw et al., 2012; Mewa et al., 2013; Plaza-Wuthrich et al., 2013).

The coefficient of variation (CV) of the traits was highest in the stressed environment. The number of productive tillers per plant had the highest CV of all the traits, in stressed and non-stressed environments, at 30.4% and 24.7%, respectively. High CVs of 28.0% and 25.4% under stressed and non-stressed condition, respectively, were observed for main shoot panicle seed weight (Abraha et al., 2016).

The overall mean grain yields of the genotypes were 1.4 and 1.0 t ha^{-1} in the non-stressed and moisture stressed environments, respectively (Abraha et al., 2016). A grain yield reduction of 29% was observed in the stressed environment compared to the non-stressed environment. Plant height and panicle length were also reduced in the stressed environment. A 25.5% grain yield reduction due to moisture stress was previously reported in tef recombinant inbred lines (Admas and Belay, 2011). Shiferaw et al. (2012) reported a greater grain yield reduction of 51 % under moisture stressed conditions for a number of tef landraces and improved varieties. Crop production in Ethiopia is rainfall-dependent with low productivity due to variable and erratic rainfall. Tef is a major crop grown across wide agro-ecologies in Ethiopia, under both optimal and moisture stressed conditions (Assefa et al., 2015). The levels of yield reductions due to moisture stress measured in these traits indicated the importance of developing moisture stress tolerant tef varieties, which could contribute to the sustainable production of tef in Ethiopia. Under optimal moisture conditions the genotypes DZ-Cr-387, 9403, 215678, 205896, Dschanger, 9415, 9432, Purpurea, DZ-01-3186 and Jano showed grain yields of above 2.0 t ha^{-1}. These genotypes were late maturing with long plant height and panicles, low lodging index, high biomass yield, harvest index and main shoot panicle seed weight. In the moisture stressed environment genotypes Dschanger, DZ-Cr-385 and DZ-Cr-37 were the top grain yielders at 1.6, 1.6 and 1.5 t ha^{-1}, respectively (Abraha et al., 2016). Overall, the results indicated that yield performance of genotypes in moisture stressed and non-stressed environments varied except for the genotype Dschanger, which had high yields at both test sites.

Genotypic and phenotypic coefficient of variation, broad sense heritability and genetic advance

Genotypic coefficient of variance (GCV) and phenotypic coefficient of variance (PCV), heritability and genetic advance (GAM) for the 11 quantitative traits of the 144 tef genotypes tested under optimal and moisture stressed environments are presented in Table 2. Highest GCV values were observed for the main shoot panicle seed weight, at 22.4% and 25.9%, and for grain yield at 17.6 % and 20.0%, in the non-stressed and stressed environments, respectively. The trends of GCV values of grain yield in the stressed and non-stressed environments observed in the present study are in agreement to the findings of Admas and Belay (2011). The PCV values were 25.9% and 20.3% for main shoot panicle seed weight and grain yield, respectively, in the non-stressed environments. Under moisture stressed conditions PCV values of 37.9%, 30.3%, 28.3% and 26.2% were recorded for main shoot panicle seed weight, harvest index, number of productive tillers per plant and grain yield, respectively. Generally, GCV ranged from 4.3 to 22.4% and 3.2 to 25.9%, while PCV varied from 4.6 to 25.9% and 4.2 to 37.9% in the non-stressed and moisture stressed environments, respectively (Table 2). These trends were in agreement to the report of Ayalew et al. (2011) in their evaluation of tef germplasm collected from Amhara region. However, the current range of variability was smaller than that reported by Chanyalew (2010), who found GCV and PCV values ranging from 4.2 to 54.5% and 10.5 to 51.0%, respectively, when testing different sets of tef genotypes at Debrezeit and Melkasa in Ethiopia.

The greatest difference between GCV and PCV values were observed in the moisture stressed environment for the majority of the traits. This was especially high for the number of productive tillers per plant, which could be attributed to the high environmental variance in the stressed environments affecting genetic variability (Table 2). Similarly, larger differences in GCV and PCV values were reported for the number of productive tiller in stressed than non-stressed environments (Admas and Belay, 2011).

Heritability of 87.1%, 78.5%, 76.0%, 75.5%, 75.0% and 70.7% were observed for days to 75% maturity, plant height, days to panicle emergence, grain yield, main shoot panicle seed weight and panicle length, respectively, under non-stressed conditions. However, heritability of 77.3%, 57.5%, 56.1% and 58.7% were estimated for days to panicle emergence, days to 75% maturity, grain filling period and grain yield respectively in the stressed environment (Table 2). Chanyalew (2010) reported an intermediate heritability of 50.5% for grain yield of tef genotypes. Similarly, Ayalew et al. (2011) recorded intermediate heritability values for days to panicle mergence (80.7%), culm length (72.4%), days to

Table 1. Testing sites and the four environment involving two water regimes used for evaluation of 144 tef genotypes during the main and off season of 2014.

Site	Environment	Treatment/water regime	Growing season	Total rainfall/irrigated water
Hastebo	E1	Optimal rainfall	Main season (July- November)	937.1
Dibdibo	E2	Optimal rainfall	Main season (July- November)	992.8
Dura	E3	Irrigated from planting till physiological maturity	Off season (January-April)	739.2
	E4	Irrigation withheld after 50% days to heading till maturity	Off season (January-April)	494.9

The rainfall amount for the rainy season at Hastebo and Dibdibo is in mm/year. The was amount applied at Dura for the irrigated experiment is in mm per crop cycle.

Table 2. Genotypic coefficients of variance (GCV) and phenotypic coefficients of variance (PCV), broad sense heritability (H) and genetic advance (GAM) as percent of mean for 11 agronomic traits of the 144 tef genotypes tested under moisture stressed and non-stressed conditions.

	Non-Stressed				Stressed			
Traits	GCV (%)	PCV (%)	H (%)	GA (% of mean)	GCV (%)	PCV (%)	H (%)	GA (% of mean)
DPE	6.9	7.9	76.0	12.3	5.6	6.8	77.3	10.8
DM	4.3	4.6	87.1	8.2	3.2	4.2	57.5	5.0
GFP	5.9	7.6	60.2	9.4	9.7	12.9	56.1	14.9
PLHT	7.3	8.2	78.5	13.2	8.1	12.1	44.7	11.1
PNLG	8.4	10.0	70.7	14.6	9.5	14.2	45.1	13.1
NPT	5.6	16.5	11.4	3.9	-	28.3	-	-
LODG	10.6	14.0	56.8	16.4	11.1	23.3	22.5	10.8
GYLD	17.6	20.3	75.5	31.5	20.0	26.2	58.7	31.6
BMYLD	8.5	12.3	47.7	12.1	6.2	20.6	8.9	3.8
HI	5.4	12.0	20.0	4.9	16.2	30.3	28.4	17.7
PSW	22.4	25.9	75.0	40.0	25.9	37.9	46.7	36.4

DPE = days to panicle emergence, DM = days to 75% maturity; GPF = grain filling period; PLHT = plant height; PNLG = panicle length; NPT = number of tillers per plant; LODG = lodging index; GYLD = grain yield; BYLD = biomass yield; HI = harvest index; PSW = main shoot panicle seed weight.

Table 3. Genotypic correlations coefficients for 11 quantitative traits of 144 tef genotypes tested in moisture stressed and non-stressed environments.

		Traits										
Traits	Env.	DPE	DM	GFP	PLHT	SPLG	NPT	LODG	GYLD	BMYLD	HI	PSW
	N	1.00										
DPE	S	1.00										
	N	0.73**	1.00									
DM	S	0.44**	1.00									
	N	-0.33**	0.41**	1.00								
GFP	S	-0.58**	0.47**	1.00								
	N	0.56**	0.70**	0.21*	1.00							
PLHT	S	0.46**	0.52**	0.02ns	1.00							
	N	0.53**	0.69**	0.25**	0.78**	1.00						
SPLG	S	0.46**	0.40**	-0.09ns	0.76**	1.00						
	N	-0.28**	-0.28**	-0.02ns	-0.31**	-0.36**	1.00					
NPT	S	-0.03ns	0.07ns	0.09ns	0.03ns	0.08ns	1.00					
	N	-0.45**	-0.52**	-0.12ns	-0.45**	-0.52**	0.06ns	1.00				
LODG	S	-0.33**	-0.20*	0.14ns	-0.32**	-0.34**	0.08ns	1.00				
	N	0.46**	0.50**	0.08ns	0.50**	0.63**	-0.30**	-0.43**	1.00			
GYLD	S	0.01ns	-0.06ns	-0.06ns	0.09ns	-0.02ns	-0.11ns	0.05ns	1.00			
	N	0.50**	0.49**	0.01ns	0.56**	0.57**	-0.25**	-0.37**	0.77**	1.00		
BMYLD	S	0.19*	0.37**	0.15ns	0.32**	0.32**	0.08ns	-0.09ns	0.24**	1.00		
	N	0.17*	0.26**	0.14ns	0.18*	0.37**	-0.24**	-0.26**	0.73**	0.14ns	1.00	
HI	S	-0.10ns	-0.30**	-0.17*	-0.13ns	-0.22**	-0.15ns	0.11ns	0.75**	-0.41**	1.00	
	N	0.47**	0.57**	0.17*	0.60**	0.66**	-0.35**	-0.34**	0.66**	0.54**	0.45**	1.00
PSW	S	0.12ns	0.35**	0.20*	0.29**	0.21**	0.00ns	-0.11ns	0.19ns	0.43**	-0.08**	1.00

DPE = days to panicle emergence, DM = days to 75% maturity; GFP = grain filling period; PLHT = plant height; PNLG = panicle length; NPT = number of tillers per plant; LODG = lodging index; GYLD = grain yield; BYLD = biomass yield; HI = harvest index; PSW = main shoot panicle seed weight; ns, * and ** indicate non-significant, significant at $p \leq 0.05$ and $p \leq 0.01$, respectively ,N and S denote Non-stressed and Stressed environments, respectively; Env. = environments.

Table 4. Direct (diagonal and bold faced scripts) and indirect (off diagonal) path coefficients of 11 quantitative traits of 144 tef genotypes tested under moisture stressed and non-stressed conditions.

		Traits										
Trait	ENV	DPE	DM	GFP	PLHT	SPLG	NPT	LODG	BMYLD	HI	SWPP	rgGYLD
	N	**-2.42**	1.81	0.61	0.00	0.01	0.00	0.01	0.33	0.11	0.01	0.46**
DPE	S	**2.31**	-0.96	-1.38	0.03	-0.02	0.00	0.00	0.13	-0.11	0.00	0.01ns
	N	-1.75	**2.50**	-0.75	-0.01	0.02	0.00	0.01	0.32	0.17	0.01	0.50**
DM	S	1.02	**-2.16**	1.13	0.03	-0.02	0.00	0.00	0.25	-0.30	-0.01	-0.06ns
	N	0.80	1.02	**-1.84**	0.00	0.01	0.00	0.00	0.00	0.09	0.00	0.08ns
GFP	S	-1.34	-1.03	**2.38**	0.00	0.00	0.00	0.00	0.10	-0.17	-0.01	-0.06ns
	N	-1.36	1.74	-0.39	**-0.01**	0.02	-0.01	0.01	0.37	0.11	0.01	0.50**
PLHT	S	1.06	-1.12	0.05	**0.06**	-0.03	0.00	0.00	0.22	-0.14	-0.01	0.09ns
	N	-1.27	1.72	-0.45	-0.01	**0.02**	-0.01	0.01	0.38	0.23	0.01	0.63**
SPLG	S	1.07	-0.87	-0.22	0.05	**-0.04**	0.00	0.00	0.22	-0.23	-0.01	-0.02ns
	N	0.67	-0.70	0.04	0.00	-0.01	**0.02**	0.00	-0.16	-0.15	0.00	-0.30**
NPT	S	-0.06	-0.14	0.20	0.00	0.00	**0.00**	0.00	0.05	-0.16	0.00	-0.11ns
	N	1.09	-1.30	0.21	0.00	-0.01	0.00	**-0.02**	-0.24	-0.17	0.00	-0.43**
LODG	S	-0.77	0.44	0.34	-0.02	0.01	0.00	**-0.01**	-0.06	0.11	0.00	0.05ns
	N	-1.21	1.22	-0.01	0.00	0.01	0.00	0.01	**0.66**	0.09	0.01	0.77**
BMYLD	S	0.44	-0.80	0.35	0.02	-0.01	0.00	0.00	**0.68**	-0.43	-0.01	0.24**
	N	-0.41	0.66	-0.26	0.00	0.01	0.00	0.01	0.09	**0.62**	0.01	0.73**
HI	S	-0.24	0.64	-0.40	-0.01	0.01	0.00	0.00	-0.28	**1.03**	0.00	0.75**
	N	-1.12	1.43	-0.31	0.00	0.02	-0.01	0.01	0.36	0.28	**0.01**	0.66**
SWPP	S	0.29	-0.76	0.46	0.02	-0.01	0.00	0.00	0.30	-0.08	**-0.03**	0.19ns

DPE = days to panicle emergence, DM = days to 75% maturity; GFP = grain filling period; PLHT = plant height; PNLG = panicle length; NPT = number of tillers per plant; LODG = lodging index; GYLD = grain yield; BYLD = biomass yield; HI = harvest index; PSW = main shoot panicle seed weight; rgGYLD = genotypic correlation of grain yield; N, and S denote for Non-stressed and Stressed environments, respectively; ENV= environments

maturity (65%), plant height (64.3%), grain filling period (61.6%) and grain yield (57.3%) for tef landraces collected from the Amhara region. Mewa et al. (2013) reported intermediate to high heritability (65.9% to 86.5%) for grain yield of recombinant inbred lines derived from a cross of the tef variety DZ-01-974 with *Eragrostis pilosa*, tested at two locations. Conversely, low heritability values of 25.3% and 25.7% were reported by Assefa et al. (2000) and Assefa et al. (2001a), respectively, for grain yield of tef landraces.

Generally, heritability was high for all traits under the non-stressed conditions but comparatively low under moisture stressed conditions (Table 2). Decreased heritability of grain yield under the stressed conditions than the non-stressed was also reported by Admas and Belay (2011) and Shiferaw et al. (2012). Information on the amount of genetic advance that could be achieved under selection is valuable in plant breeding programs. Main shoot panicle seed weight with 40.0% and 36.4% followed by grain yield of 31.5% and 31.6% had higher rates of genetic advance in the non-stressed and stressed environments, respectively (Table 2). High genetic advance translates to more progress from plant breeding selection. Assefa et al. (1999) and Ayalew et al. (2011) reported a genetic advance of 24.7% and 24% respectively, for grain yield of tef landraces. Chanyalew (2010) estimated a genetic advance of 31.3% and 47.9% for grain yield and main shoot panicle seed weight, respectively. In contrast, Assefa et al. (2001a) indicated a low genetic advance of 14.5% for grain yield of tef landraces collected from eight regions in Ethiopia.

Correlations of yield and its components

Genetic correlation coefficients among the 11 traits grown under non-stressed and stressed conditions are presented in Table 3. Grain yield showed significant ($p \leq 0.01$) positive correlation with biomass yield (r=0.77), harvest index (0.73) and main shoot panicle seed weight (0.66) in the non-stressed environments. Days to panicle emergence (0.46), days to maturity (0.50), plant height (0.50) and panicle length (0.63) were also positively associated with grain yield. Under moisture stressed condition, grain yield showed significant association with biomass yield (0.24) and harvest index (0.75) only (Table 3). This correlation could be due to linkage or pleiotropic genetic effects causing the traits to change in the same direction (Falconer and Mackay, 1996). Chanyalew (2010) reported positive correlation of grain yield with the majority of the traits tested, while negative correlations were recorded with harvest index and lodging index for tef genotypes tested at two locations. Lule and Mengistu (2014) reported a positive association of grain yield with harvest index but a negative association with biomass yield of tef landraces collected from different zones of Ethiopia. Plant height and panicle length showed positive associations with grain yield. However, lodging index was negatively correlated with these traits (Table 3). Interestingly, this may not translate into a high harvest index and reduced lodging, which have been reported as important traits of semi-dwarf varieties of small cereals such as wheat, barley and rice. Therefore, development of considerably dwarf tef varieties should not be the goal of tef breeding to enhance grain yields and reduce effect of lodging. In Ethiopia, relatively tall tef varieties are desired by farmers because tef is highly valued for its straw yield as a major source of animal feed (Yami, 2013). Tall tef varieties have relatively thick stems. Tef has a shallow root system and is sensitive to lodging (Van Delden et al., 2010). Late maturing and tall tef varieties possess deeper root systems than early maturing genotypes that have shorter plant heights (Ayele et al., 2001). Therefore, breeding tef varieties with a good stem thickness and improved root depth could offer high adoption rate of tef varieties by farmers than breeding dwarf varieties to reduce lodging. Number of productive tillers per plant revealed a significant ($p \leq 0.01$) negative association (r = -0.30) with grain yield in the non-stressed environment and a non-significant negative association (r=-0.11) in the stressed environment (Table 3). Unlike these findings, Ayalew et al. (2011) reported a positive correlation between the number of productive tillers per plant with grain yield. Days to 75% maturity was positively associated with grain yield (r=0.50) in the non-stressed environment, while there was a non-significant negative association (r=-0.06) in the stressed environment, supporting the importance of early maturity in moisture stressed environments (Table 3). Similarly, Tefera et al. (2003) and Plaza-Wuthrich et al. (2013) found negative correlations between grain yield and days to maturity in tef recombinant inbred lines, and landraces, respectively.

Path coefficient analysis

Table 4 presents the direct and indirect effect of the 11 quantitative traits on grain yield under non-stressed and moisture stressed conditions. Biomass yield and harvest index showed strong positive effects of 0.66, 0.68 and 0.62, 1.03 on grain yield in both the non-stressed and stressed environments, respectively (Table 4). Therefore, selection for these characters would give good responses to yield improvement. Previously, Ayalew et al. (2011) reported biomass yield, number of productive tiller per plant and harvest index for their highest direct effect and their correlation with grain yield of tef landraces. The authors suggested that selecting for these traits indirectly selects for grain yield. Similarly, Ayalneh et al. (2012) and Debebe et al. (2013) reported that harvest index and biomass yield had a strong direct effect and positive correlation with grain yield in tef landraces.

Days to panicle emergence and grain filling period showed strong negative direct effects of -2.42 and -1.84 under the non-stressed conditions and strong positive direct effects of 2.31 and 2.38 under stressed conditions. Convserly, days to maturity had a strong positve direct effect in the non-stressed environment (2.50) and a strong negative effect in the stressed enviroment (-2.16). This indicates that late maturity tends to decrease grain yield performance during drought stress. Similarly, Admass and Belay (2011) found better grain yield performance of early maturing recombinant inbred lines than late maturing types in a moisture stressed environment. In addition to its direct effect, days to maturity showed relatively strong negative indirect effects via grain filling period, plant height, panicle length, number of productive tillers per plant, biomass yield and main shoot panicle seed weight in the stressed environment, and positive indirect effects via all traits except lodging index and number of productive tillers per plant in the non-stressed environments (Table 4).

Plant height and panicle length exerted weak direct effects in both environments. A significant positive association of these traits with grain yield under the non-stressed conditions was due to positive indirect effects via biomass yield and days to maturity. Similarly lodging index, number of productive tillers per plant and main shoot panicle seed weight had minimal direct effects under both environments (Table 4). Overall, the path analysis indicated selection for high biomass yield, harvest index and long maturity could provide increased grain yield in optimal environments. While in moisture stressed environments, yield improvement could be achieved through selection for reduced days to maturity, high biomass yield and harvest index.

Materials and Methods

Description of the study environments

The study was conducted at three sites in Tigray region of northern Ethiopia, namely: Dura ($14^0$06'76.2"N, $038^0$39'14.5"E, 2073 meter above see eve (m.a.s.l.)), Hastebo ($14^0$06'40.2"N, $038^0$45'45.8"E, 2118 m.a.s.l,) and Dibdibo ($14^0$16'22.1"N, $039^0$04'15.6"E, 2014 m.a.s.l.) which are on-farm research sites of Axum Agricultural Research Center (AxARC). Dura is an irrigation potential which was suitable to conduct offseason experiment, while at Hastebo only the main season experiment was conducted following the rainy season. The 13 years data of the testing sites shows the variable and erratic trend of rainfall across the years (Ethiopian Meteorology Agency, 2014). The rainfalls at Hastebo and Dibdibo during 2014 were 937.1 and 992.8 mm/year, respectively (Table 1), which were optimal conditions for tef production. The rainfall distribution was high during July and August but considerably lower during the grain filling periods (September –November) (Abraha et al., 2016). The mean minimum and maximum temperatures at Dura and Hastebo sites varied from 9.9-29.4^{o}C, while that of Dibdibo ranged from 7.72 -36.11 ^{o}C (Abraha et al., 2016). The soil types are clay at Dura, clay loam at Hastebo and sandy loam at Dibdibo.

Plant materials

The test entries included 144 tef genotypes which are listed in Supplemental Table 1 along with their site of collection. Of these about 92 accessions were collected from six administrative zones of Tigray region, namely; north-west, south-east, central, south, east and west. These germplasm were sourced from the Ethiopian Biodiversity Institute (EBI (Supplemental Table 1). In addition 32 improved tef varieties were included, which had been released since 1970 in Ethiopia. The remaining test genotypes comprised 18 of the 35 tef landrace cultivars described by Ebba (1975) and were originally collected from the Shoa (8), Gojam (3), Keffa (2), Welega (3) and Hararge (2) regions in Ethiopia.

Experimental design and trial management

The trial was laid out as a 12 x 12 simple lattice design with two replications. Each genotype was planted in four 1 m long rows with inter-row spacing of 0.25m. Seeds at the rate of 15 kg ha^{-1} were drilled in the four rows. The plot size and design was decided based on the number of genotypes and the availability of uniform land to minimize experimental error. Fertilizers in the form of Diammonium phosphate (DAP) and Urea were each applied at 100 kg ha^{-1} with the DAP applied entirely at planting time, while urea was split applied (half during emergence and the remaining half four weeks after planting) according to the recommendation made by AxARC (2011), for efficient utilization of the fertilizer by the plants. Weeding was manually done twice, at three weeks and five to six weeks after planting. Harvesting for data collection was done from the two central rows of each plot.

Across the three testing sites four experiments were conducted involving two water regimes: Regime I (optimal moisture) at Hastebo (environment 1) and Dibdibo (environment 2) (main season rainfall, July - November 2014) and irrigated from planting till physiological maturity (off-season, January to April 2014) at Dura site (environment 3; and regime II (water-stressed) at Dura site (environment 4) with irrigation withheld after 50% days to heading till maturity during the off-season (January-April 2014 (Table 1). The irrigated experiment was used to evaluate the performance of genotypes under moisture stressed and non-stressed (control) condition, which could not be done under rain fed conditions, because of the difficulties to control water treatment.

Crop water requirement was calculated using the following formula: CWR = KC x ETo, where CWR is crop water requirement, KC is crop coefficient determined at different growth and developmental stages and ETo is reference crop evapotranspiration. ETo was calculated using CROPWAT 8.0 software developed by FAO (FAO, 1998) developed by the Land and Water Division of FAO. Climate data including daily rainfall, maximum and minimum temperature, relative humidity, sunshine hours and wind speed of Dura site were obtained from Ethiopian meteorological Agency of Mekelle branch to calculate ETo. A total of 739.2 mm and 494.9 mm irrigated water were applied under the non-stressed and moisture stressed conditions, respectively (Table 1). The required amount of water needed to be applied for a plot was measured using partial flow.

Data collection

The following data were collected, based on a whole plot basis, measuring the two central rows: days to panicle emergence, days to 75% maturity, grain filling period as the difference between days to 75% maturity and days to panicle emergence, grain yield (g), biomass yield (g), harvest index and lodging index. The later was scored according to the procedure of Caldicott and Nuttall (1979) for each plot using a 0-5 scale, where 0 indicates plants in an upright position and 5 for plants lying flat on the ground. The lodging index was then calculated as the mean of the product sum of the angle of lodging and the corresponding percentage. In addition, individual plant parameters based on 10 randomly selected plants from the two central rows of each plot were recorded at maturity for plant height (cm), panicle length (cm), number of productive tillers per plant and main shoot panicle seed weight.

Data analysis

Data collected across the three environments; under two optimal rainfall and a full irrigation regime were regarded as non-stressed environments and the other test involving irrigation being withheld at panicle emergence being considered as moisture stressed environment. Data of the 144 genotypes, grown in the non-stressed and stressed environments, were subjected to statistical analysis using the simple lattice procedure of SAS 9.3 (SAS Institute 2011). Combined analyses of variance over non-stressed environments were carried out after homogeneity of variance test procedure. The expected mean squares from the analysis of variance were used to estimate the phenotypic coefficient of variance (PCV) and genotypic coefficient of variance (GCV), heritability (H) and genetic advance (GAM). Associations among the 11 traits were assessed, based on means, using the Pearson's correlation procedure of SAS 9.3 (SAS Institute 2011). Path analysis was conducted according to the procedure by Dewey and Lu (1959).

Conclusion

The current study indicated that the existence of substantial genetic variability within tef genotypes screened in optimal and moisture stressed environments useful for tef improvement. Heritability values of traits were higher in the optimal environments than they were in the moisture stressed environment due to the relatively high environmental variability. The relatively high genetic advance of grain yield and main shoot panicle seed weight under both optimum and moisture stressed conditions would be desirable for genetic gains through selection. Direct selection for high biomass, harvest index and late maturity could increase grain yield in optimal environments, while under moisture stress conditions early maturity, high biomass and harvest index were important direct selection criteria to use when breeding tef for drought tolerance. Due to the limited number of stressed environments used in the present study, further evaluation of the genotypes across more representative and water stressed agro-ecologies would be useful.

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