

Evaluation of durum wheat genotypes (*Triticum turgidum* L. var. *durum*) for drought tolerance

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Abstract: Understanding genotypic differences of drought response and related, morphological and physiological traits in major crops such as durum wheat is paramount for meaningful crop improvement. Durum wheat remains a critical staple food in marginal land due to its exceptional adaptation to climatic stresses. Nonetheless, its production is threatened and limited due to increasing unpredictable changes in climatic elements such as rainfall and severe drought. In this study, we aimed to identify agronomic, morphological, and physiological traits, and stress tolerance indices most suitable to characterize the drought response of durum wheat genotypes sourced from Ethiopia. For this, we conducted a drought experiment with 15 genotypes of durum wheat. Drought and well-watered treatments were set based on field capacity (FC) of the soil. We found that drought significantly affected plant height, spike length, number of grains per spike, leaf area per plant, biomass and grain yield. Variability in grain yield, plant height, and number of grains per spike across different moisture regimes were observed, but the response patterns remained the same across genotypes, particularly under drought stress. Strong correlations were observed between yield under drought stress and stress tolerance indices. Principal component analysis showed that 65% of the total variation was explained by the first two principal components (PCs) under drought stress. Thus, traits included in the first PC, particularly traits with higher vector loading scores (spike length, number of grains per spike, grain yield and harvest index) and stress tolerance indices are pertinent in screening durum wheat genotypes under drought stress.

Keywords: Field capacity of the soil; Principal component; Stress tolerance indices.

Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is one of the ten most important crops in the world with an annual production of 37 million tonnes (Kabbaj et al., 2017). 6.2% of the world's wheat acreage is planted with this species of allotetraploid wheat, which is the most widely grown (Martínez-Moreno, 2022). With 0.6 million hectares (FAOSTAT, 2015; Sall et al., 2019), Ethiopia is the largest producer of durum wheat in Sub-Saharan Africa (SSA). Roughly 30% of arable land and 15-20% of the wheat production in Ethiopia are devoted to durum wheat (Negassa et al., 2013; Alemu et al., 2019). It has grown to be a significant cash crop in the country with prices 10–20% higher than those of bread wheat, in addition to being a staple crop for food security (Sall et al., 2019).

Wheat accounts for about 17% of total grain production in Ethiopia making it the third most important cereal crop after teff [*Eragrostis tef* (Zucc.) Trotter] and maize (*Zea mays* L.) (CSA, 2021). However, its production is heavily dependent on the water coming from rainfall. Furthermore, the trend of rainfall is likewise altering as a result of the shifting global climate (Funk et al., 2012; Stroosnijder et al., 2012). This poses a serious risk to the productivity and production of durum wheat. Hence, testing of crop genotypes for drought tolerance using agronomic and physiological traits to drought stress may serve as a potent approach to screen and develop new cultivars (Raza et al., 2020). However, this requires a deeper comprehension of the process that determines yield (Blum and Pnuel, 1990).

Climate change scenarios predict an increase in precipitation variability in the future, and certainly water will become increasingly scarce. Alarming, the percentage of drought affected land has approximately doubled from the 1970s to the early 2000s, affecting grain yield and quality of various crops resulting in food shortages in the world (Isendahl and Schmidt, 2006). Durum wheat productivity in Ethiopia particularly in mid-altitude marginal soil such as Vertisol, having a swell and shrink property were low and this soil is also known to induce water scarcity during dry spell and waterlogging during wet season. Usually, farmers in these areas adopt a strategy through late wet season sowing viz. August sowing of this crop is to avoid waterlogging problems. Since the water holding capacity of Vertisol is very high, there will be enough water held in the soil to grow the crop with mild rain afterward. However, seasonal variability in rainfall in these areas and even in high potential durum wheat producing areas is under heightened risk and challenges of drought impacts. Thus, it requires understanding and elucidating the impacts of drought on different durum wheat genotypes to identify the best thriving genotypes that could be put into the pipeline for release and/or used in a variety development program for drought tolerance. Identifying agronomic, morphological and physiological traits and the associated stress tolerance indices which best characterize drought response of durum wheat is paramount.

Nonetheless, so far most of the durum wheat breeding programs in the country have given more emphasis on yield, disease resistance, and to some extent on quality-related traits under ideal moisture conditions (non-drought stress). This has led to a few durum wheat varieties have been developed for drought tolerance in combination with optimized yields or quality. So, understanding the underlying mechanism of drought tolerance and evaluating the traits in durum wheat genotypes is necessary. Also, related traits other than crop yield and quality have been less studied which could substantiate the selection process for drought tolerance. Hence, looking into stress tolerance indices, stomata morphology and physiological traits including grain yield and yield related traits might be a good strategy to evaluate genotypes under drought stress conditions.

Therefore, the objective of the current study was to evaluate 15 durum wheat genotypes sourced from Ethiopia exposed to drought stress based on field capacity (FC) of the soil by focusing on different growth traits, yield and yield related traits as well as stress tolerance indices to identify effective traits to facilitate screening of durum wheat genotypes for breeding programs focusing on better yield performance under drought stress conditions.

Results

Spike length, stomata number & leaf area per plant

Moisture regime significantly affected spike length, stomata number (square root transformed) and total leaf area per plant (Table 1). Drought reduced spike length by 1.22 cm but increased stomata number by 15.6% compared to stomata number under well-watered conditions (Table 1). Drought has resulted in a 38.7% reduction of leaf area per plant (Table 1). Drought effects on specific leaf area and leaf area ratio were not significant (Table S2).

Table 1. Main effect of moisture regimes for spike length, stomata number and leaf area per plant.

Moisture regimes	Spike length (cm)	SN_sq. root	Leaf area per plant (cm ²)
80% of FC	4.94±0.07 ^a	7.04±0.21 ^b	16.06±0.95 ^a
30% of FC	3.72±0.07 ^b	8.14±0.24 ^a	9.84±0.61 ^b
HSD Tukey's	***	**	***

SN_sq.root is square root transformed value of stomata number. Least square mean ± (Standard error), values with different superscripted letters are significantly different according to Tukey's HSD test (P < 0.05). **P < 0.01; ***P < 0.001.

Number of grains per spike

Analysis of variance showed a highly significant effect (P < 0.001) of genotypes by moisture regimes interaction on number of grains per spike (Table S1). Our results showed that, in the case of well-watered conditions compared to the drought tolerant check (G8), all of durum wheat genotypes evaluated had statistically similar numbers of grain per spike (Table 2). However, G12 had a significantly lower number of grains per spike than G9 (Table 2). In contrast, under drought stress conditions, there were no significantly noticeable differences among genotypes with regard to number of grains per spike (Table 2). To provide explanation on the importance of the interaction of genotypes by moisture regimes on number of grains per spike, under drought conditions, there was no statistically significant difference among the genotypes considered in this study implying that the interaction of genotypes by moisture regimes was vital under well-watered conditions.

Grain yield

Results of the analysis of variance of grain yield per pot data showed a very highly significant genotypes × moisture regimes (G×MR) interaction (P<0.001, Table S1). Variability in grain yield across different moisture regimes was observed, but the response patterns remained the same across genotypes, particularly under drought stress. The mean yields of genotypes under drought stress ranged from 0.05 g pot⁻¹ (G4) to 1.08 g pot⁻¹ (G13), whereas the mean yield of genotypes under well-watered conditions ranged from 0.98 g pot⁻¹(G8) to 2.48 g pot⁻¹(G11) (Table 2).

The ranking of genotypes according to grain yield per pot was not different, indicating the responses pattern of genotypes to different levels of moisture regimes remain the same particularly under drought stress conditions. Thus, compared to the

drought tolerant genotype (G8), all of the genotypes evaluated had statistically at par grain yield per pot under drought stress conditions (Table 2). Even under well-watered conditions, with the exception of genotypes G11 and G9 which significantly out yielded genotype G8, the rest of genotypes produced similar grain yield per pot. In contrast, G11 and G9 had 153.1% and 137.8% respective increments over the drought tolerant check Alem-Tena (G8) under well-watered conditions (Table 2). When we look at the relative traits change for grain yield compared to their counterparts under well-watered conditions, the lowest reduction was observed from G8 (10.2%) followed by G13 (44.04%) and G10 (52.2%) whereas the highest reduction was recorded from G4 (95%), followed by G3 (88.7%) and G15 (85.6%) (Table 2).

Table 2. Responses of durum wheat genotypes under different moisture regimes on number of grains per spike and grain yield.

Genotypes	Number of grains per spike			Grain yield/pot (g)		
	Moisture regimes (FC)		Relative traits change (%)	Moisture regimes (FC)		Relative traits change (%)
	80% of FC	30% of FC		80% of FC	30% of FC	
G1	13.72 ±0.90 ^{ab}	4.33±1.31 ^a	68.44	1.18±0.15 ^b	0.22±0.08 ^{ab}	81.36
G2	19.22 ±1.39 ^{ab}	4.70 ± 1.91 ^a	75.55	1.70±0.09 ^{ab}	0.27±0.09 ^{ab}	84.12
G3	16.57± 1.69 ^{ab}	1.42 ± 0.95 ^a	91.43	1.5±0.10 ^{ab}	0.12±0.06 ^{ab}	88.67
G4	13.32± 1.35 ^{ab}	1.65 ± 0.57 ^a	87.61	1.00±0.13 ^b	0.05±0.03 ^b	95.00
G5	11.68± 0.82 ^{ab}	5.18 ± 0.57 ^a	55.65	1.28±0.20 ^b	0.35±0.08 ^{ab}	72.66
G6	19.47± 1.24 ^{ab}	8.28 ± 2.09 ^a	57.47	1.68±0.19 ^{ab}	0.60±0.04 ^{ab}	64.29
G7	14.45± 1.54 ^{ab}	3.85 ± 1.60 ^a	73.36	1.75±0.13 ^{ab}	0.53±0.22 ^{ab}	69.71
G8†	12.23± 1.41 ^{ab}	10.37 ± 0.95 ^a	15.21	0.98±0.15 ^b	0.88±0.10 ^{ab}	10.20
G9	20.17± 0.87 ^a 11.68±	6.58 ± 2.45 ^a	67.38	2.33±0.18 ^a	0.68±0.25 ^{ab}	70.82
G10	1.08 ^{ab} 17.33±	7.03 ± 0.90 ^a	39.81	1.15±0.11 ^b	0.55±0.11 ^{ab}	52.17
G11	0.83 ^{ab} 13.83±	8.15 ± 1.21 ^a	52.97	2.48±0.12 ^a	0.45±0.12 ^{ab}	81.85
G12	10.67± 1.47 ^b 13.83±	3.28 ± 0.95 ^a	69.26	1.50±0.10 ^{ab}	0.23±0.10 ^{ab}	84.67
G13	0.74 ^{ab} 16.30±	6.05 ± 1.23 ^a	56.25	1.93±0.16 ^{ab}	1.08±0.11 ^a	44.04
G14	2.30 ^{ab} 15.92±	4.38 ± 1.16 ^a	73.13	1.32±0.11 ^b	0.50±0.15 ^{ab}	62.12
G15	0.48 ^{ab}	2.05 ± 0.77 ^a	87.12	1.18±0.18 ^b	0.17±0.08 ^{ab}	85.59
Tukey's HSD	***			***		

Note: Least square mean ± (Standard error), values with different superscripted letters are significantly different according to Tukey's HSD test (P < 0.05). ns, not significant; * P < 0.05; **P < 0.01; ***P < 0.001. † Drought tolerant check.

Plant height

ANOVA revealed that there was highly significant (P<0.01) interaction between genotypes and moisture regimes (Table S1). Although, there was highly significant interaction. The plant height was not a desirable trait in screening genotypes under drought stress conditions in the case of our study.

Under well-watered conditions, maximum plant height was recorded in genotype G3 (87.8 cm) while minimum was observed in G4 (64.7cm) (Figure 1), and under well-watered conditions compared to the drought tolerant check genotype (G8; 65.2 cm), G3 (87.8 cm) and G7 (81.5 cm) had significantly higher plant height (Figure 1). Whereas the remaining genotypes evaluated were not significantly different in plant height compared with G8 (Figure 1).

In the case of drought stress conditions, compared to drought tolerant genotype (G8), none of the genotypes considered in this study were significantly taller than the drought tolerant check (G8) (Figure 1). Thus, our result shows, plant height is not useful trait in the screening of genotypes for drought tolerance.

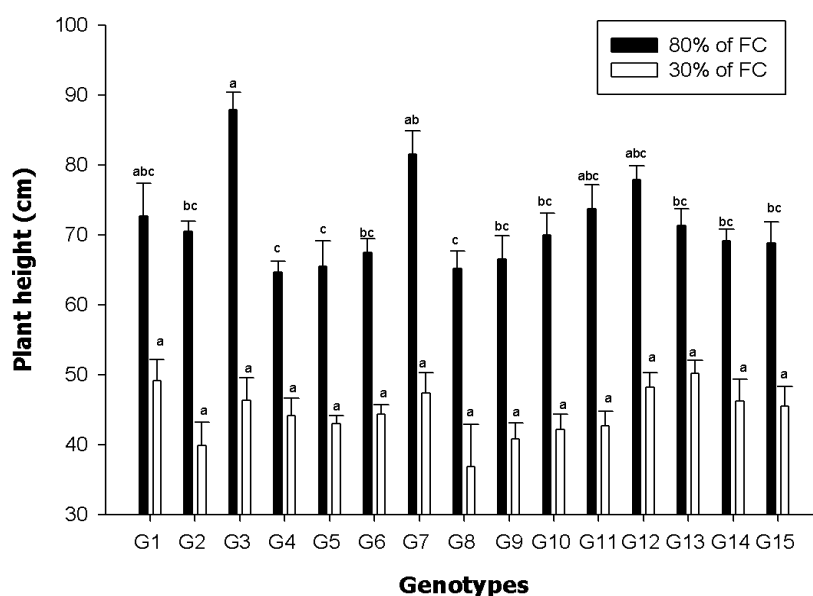


Figure 1. Plant height of durum wheat genotypes under two moisture regimes. Least square means followed by a different letter indicates significant differences at $P < 0.05$ according to Tukey's HSD test.

Biomass yield

Biomass yield per pot was very highly significantly affected by moisture regimes and durum wheat genotypes ($P < 0.001$), but not by their interaction (Table S1). Biomass yield was drastically reduced under drought conditions with 79.2% (Table 3). Under both well-watered and drought stress conditions, the biomass yield per pot was higher for durum wheat genotypes G3 and G15 with respective increments of 25.9% and 26.2% compared to drought tolerant check (G8) (Table 3). On the other hand, under both moisture regimes, genotypes such as G1, G4, G5, G7, G9, G11 and G12 had statistically at par biomass yield per pot with the highest performing genotypes G3 and G15 (Table 3). In general, drought stress causes a severe decline in plant biomass per pot. Also, there was variability in biomass yield among the genotypes evaluated irrespective of moisture regimes.

Table 3. Main effect of moisture regimes and genotypes on biomass yield.

Moisture regimes	Biomass yield/pot (g)
80% of FC	4.30±0.08 ^a
30% of FC	2.40±0.06 ^b
HSD Tukey's	***
Genotypes	
G1	3.31±0.30 ^{ab}
G2	3.13±0.38 ^{bc}
G3	3.94±0.46 ^a
G4	3.62±0.39 ^{ab}
G5	3.56±0.32 ^{ab}
G6	3.10±0.32 ^{bc}
G7	3.56±0.34 ^{ab}
G8 †	3.13±0.27 ^{bc}
G9	3.44±0.27 ^{ab}
G10	2.56±0.23 ^c
G11	3.28±0.35 ^{abc}
G12	3.68±0.40 ^{ab}
G13	3.13±0.32 ^{bc}
G14	3.09±0.25 ^{bc}
G15	3.95±0.36 ^a
Tukey's HSD	***

Note: Least square mean ± (Standard error), values with different superscripted letters are significantly different according to Tukey's HSD test ($P < 0.05$). ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. † Drought tolerant check.

Stress tolerance indices

Under well-watered conditions, stress tolerance indices were significantly but moderately correlated with grain yield, HM ($r = 0.60, p < 0.001$), GMP ($r = 0.70, p < 0.001$), and YI ($r = 0.39, p < 0.05$) (Table 4). Whereas TOL ($r = 0.74, p < 0.001$), MPI ($r = 0.89, p < 0.001$), and STI ($r = 0.74, p < 0.001$) were significantly and strongly correlated with grain yield under well-watered conditions (Table 4). In addition, yield stability index (YSI) was the only index which was found to be negatively correlated with grain yield under well-watered conditions while the other indices considered in this study were positively correlated with grain yield under well-watered conditions.

Under drought stress conditions, stress tolerance indices such as TOL and SSI were significantly and negatively correlated with grain yield under drought. TOL ($r = -0.32, p < 0.05$) showed a weak negative correlation with grain yield under drought while SSI ($r = -0.71, p < 0.001$) featured a strong negative correlation with grain yield under drought stress conditions (Table 4). On the other hand, stress tolerance indices, MPI ($r = 0.77, p < 0.001$), HM ($r = 0.96, p < 0.001$), GMP ($r = 0.91, p < 0.001$), YI ($r = 0.98, p < 0.001$), YSI ($r = 0.71, p < 0.001$), and STI ($r = 0.87, p < 0.001$) showed positive and highly significant correlation with grain yield under drought conditions (Table 4). Hence, those stress tolerance indices which had strong significant correlation with grain yield under drought stress conditions could be used as screening criteria for durum wheat genotypes for drought tolerance.

Table 4. Pearson's correlation among stress tolerance indices and yield under well-watered and drought stress conditions.

	Stress tolerance indices							
	TOL	MPI	HM	GMP	SSI	STI	YSI	YI
Y_p	0.74***	0.89***	0.60***	0.70***	0.24 ^{ns}	0.74***	-0.24 ^{ns}	0.39*
Y_s	-0.32*	0.77***	0.96***	0.91***	-0.71***	0.87***	0.71***	0.98***

*, **, *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$ probability levels respectively; ns= not significant, TOL= Tolerance index, MPI= Mean productivity index, GMP= Geometric mean productivity index, HM=Harmonic mean, STI= Stress tolerance index, SSI= Stress susceptibility index, YSI= Yield stability index, YI= Yield index, Y_p is yield under well-watered conditions and Y_s is yield under drought stress conditions.

Mean value of stress tolerance indices

Mean value for stress tolerance indices were computed and ranked (Table 5). The lowest tolerance index (TOL) was recorded for genotypes, G10 (0.23) and G8 (0.28) (Table 5). The MPI genotypes, with higher MPI values were regarded as tolerant genotype and accordingly genotypes G6 (0.98), G12 (0.99), G2 (1.04), G14 (1.10), G7 (1.38), G13 (1.53), G11 (1.58), and G9 (1.68). They had higher values of MPI compared to the tolerant check G8 (0.89) (Table 5). As to the HM, genotypes G6 (0.87), G10 (0.87), G14 (0.99), G11 (1.08), G7 (1.27), and G9 (1.52) had higher HM values than the drought tolerant check G8 (0.80). Regarding the geometric mean productivity index (GMP), genotypes G10 (0.87), G6 (0.92), G14 (1.04), G11 (1.30), G7 (1.32), G13 (1.47), and G9 (1.60) were found to have high values compared to the drought tolerant check Alem-tena (G8; 0.85) (Table 5). As to the stress susceptible index (SSI), all genotypes except for genotypes G2 (1.17), G3 (1.36), G4 (1.55), G11 (1.14), G12 (1.24), G15 (1.33) had < 1 SSI value and none of the genotypes considered in this study had lower SSI value than the drought tolerant check (G8; 0.16) (Table 5). Moreover, genotypes G6, G7, G9, G10, G11, G13 and G14 with stress tolerance index (STI) values of 0.39, 0.89, 1.19, 0.35, 0.78, 1.00 and 0.50, respectively were the most stable genotypes under drought stress conditions (Table 5). Regarding yield index (YI), genotypes G7, G9, G10, G13 and G14 with respective YI values of 1.82, 2.25, 1.41, 2.24, and 1.42 were the most stable genotypes (Table 5).

Under drought stress conditions, grain yield significantly correlated with HM ($r = 0.96***$), MPI ($r = 0.77***$), GMP ($r = 0.91***$), STI ($r = 0.87***$), YSI ($r = 0.71***$) and YI ($r = 0.98***$) (Table 4). In contrast, TOL ($r = -0.32**$) showed a weak negative correlation with grain yield under drought stress condition, whereas SSI ($r = -0.71***$) had strong negative but highly significant correlation with grain yield under drought stress conditions (Table 4). Hence, among genotypes evaluated, genotypes G6, G7, G9, G10, G11, G13 and G14 were identified as drought resistant genotypes and desirable under drought stress condition.

Principal component analysis

Under well-watered conditions, three principal components (PCs) were identified with Eigenvalues ranging from 1.38 to 2.33. The first PC was the most important as it accounted for 32.9% of the total variation (Table 6). In this PC1, variables contributing with significant Eigen vector loading were NGPS (0.498), GY (0.538) and HI (0.582) (Table 6).

The second principal component accounted for 25.3% of the total variability. Accordingly, plant height (-0.434) and spike length (-0.379) had negative significant coefficients whereas number of ears per plant (0.626) and grain yield (0.372) had positive coefficients/Eigen vector loadings (Table 6). PC3 accounted for 19.6% of the total variation, which was contributed mostly by biomass yield (0.745), spike length (0.428), and plant height (0.370) variables (Table 6). All traits showed positive contribution.

Table 5. Mean values of stress tolerance indices of tested Ethiopian durum wheat genotypes.

Genotype	TOL	MPI	HM	GMP	SSI	STI	YSI	YI
G1	0.63	0.68	0.52	0.59	0.99	0.17	0.38	0.69
G2	1.22	1.04	0.67	0.83	1.17	0.32	0.27	0.80
G3	1.27	0.87	0.40	0.59	1.36	0.17	0.15	0.43
G4	1.23	0.65	0.06	0.12	1.55	0.02	0.03	0.06
G5	0.50	0.78	0.66	0.71	0.60	0.24	0.63	0.99
G6	0.62	0.98	0.87	0.92	0.74	0.39	0.54	1.24
G7	0.75	1.38	1.27	1.32	0.68	0.81	0.57	1.82
G8 †	0.28	0.89	0.80	0.85	0.16	0.33	0.89	1.37
G9	0.95	1.68	1.52	1.60	0.68	1.19	0.57	2.25
G10	0.23	0.88	0.87	0.87	0.38	0.35	0.76	1.41
G11	1.75	1.58	1.08	1.30	1.14	0.78	0.29	1.31
G12	1.22	0.99	0.59	0.75	1.24	0.30	0.23	0.72
G13	0.65	1.53	1.43	1.47	0.53	1.00	0.68	2.24
G14	0.65	1.10	0.99	1.04	0.69	0.50	0.57	1.42
G15	0.64	0.48	0.23	0.29	1.33	0.06	0.18	0.31

†Drought tolerant check; Alem-tena. TOL= Tolerance index, MPI= Mean productivity index, GMP= Geometric mean productivity index, HM=Harmonic mean, STI= Stress tolerance index, SSI= Stress susceptibility index, YSI= Yield stability index, and YI= Yield index,

Table 6. Eigenvectors, eigenvalues and cumulative percent of variation of the first three principal components for seven traits of durum wheat genotypes under well-watered conditions.

Variable	PC1	PC2	PC3
SL (cm)	0.2372	-0.3790	0.4281
NGPS	0.4982	-0.0864	0.1483
BM (g)	-0.1670	0.2884	0.7447
GY (g)	0.5384	0.3723	0.0917
NEPP	-0.1296	0.6261	0.2147
PH (cm)	0.1508	-0.4339	0.3700
HI (%)	0.5816	0.2172	-0.2208
Eigenvalue	2.3292	1.7875	1.3840
Total variation (%)	0.3292	0.2526	0.1956
Cumulative total variation (%)	0.3292	0.5818	0.7775

Note: SL, spike length; NGPS, number of grains per spike; BM, Biomass yield; GY, grain yield; NEPP, number of ears per plant; PH, plant height and HI, harvest index.

In case drought stress conditions, two PCs with Eigenvalues > 1 were identified (Table 7). The two PCs explained a cumulative 64.6% of the total variation observed in the data (Table 7). The first PC accounted for 47.2% of the total variation, which was mainly contributed by spike length (0.337), number of grains per spike (0.463), grain yield per pot (0.505) and harvest index (0.512) (Table 7). Moreover, in the first PC, all of the significant attributes were positively contributed. The second PC contributed 17.4% of the total variation, which was chiefly associated with traits such as biomass yield per pot (0.602), number of ears per pot (0.667) and plant height (0.329) (Table 7).

In general, attributes included in the first principal component were particularly traits with significant vector loading scores might be vital in screening durum wheat genotypes under drought stress conditions.

Table 7. Eigenvectors, eigenvalues and cumulative percent of variation of the first two principal components for seven traits of durum wheat genotypes under drought stress conditions.

Variable	PC1	PC2
SL (cm)	0.3372	0.2712
NGPS	0.4631	-0.0488
BM (g)	-0.2633	0.6017
GY (g)	0.5052	0.0607
NEPP	-0.0029	0.6673
PH (cm)	0.2923	0.3291
HI (%)	0.5117	-0.0689
Eigenvalue	3.3039	1.2167
Total variation (%)	0.4722	0.1739
Cumulative total variation (%)	0.4722	0.6460

Note: SL, spike length; NGPS, number of grains per spike; BM, Biomass yield; GY, grain yield; NEPP, number of ears per plant; PH, plant height and HI, harvest index.

Discussion

The variability among 15 durum wheat genotypes was evaluated under well-watered and drought stress conditions. Differences in agronomic performance are indicative of genetic variation, which is the basis for crop improvement (Seher et al., 2015; Ayalew et al., 2016). Presence of genetic variability among the test genotypes for traits related to stress tolerance is paramount for successful breeding aimed to develop cultivars adapted to a range of stress environments (Sharma et al., 2014; Mwadzingeni et al., 2017; Mathew et al., 2018). Our analysis revealed that moisture regimes, genotypes and the interaction of both factors had a significant effect on most of the studied traits.

In our study, spike length, total leaf area per plant, and biomass yield were considerably affected by drought stress. This suggests that the differences among genotypes were consistent across all moisture regimes in the case of biomass yield. Concomitant with this study, Hasan and Tacettin (2010) stated that drought stress decreased several traits including plant height, number of spikes per m², peduncle length, spike length, number of grains per spike, grain yield, and 1000 grain weight of wheat genotypes. Also, it is evident that leaf area is the most affected parameter by drought stress even under mild water stress. Most studies confirmed that wheat tends to reduce its leaf area in response to drought stress (Kam et al., 2007 and Nezhadahmadi et al., 2013). Likewise, a study was performed to determine the effect of drought stress on several physiological and agro-morphological traits in 17 durum wheat genotypes under two moisture regime conditions (well-watered and drought) over two years (Pour-Aboughadareh et al., 2020). The study showed that drought stress significantly reduced the grain filling period, plant height, peduncle length, number of grains per spike, 1000 seed weight, grain yield, biomass yield and harvest index in all wheat genotypes compared to the well-watered conditions.

In contrast, square root transformed value of stomata number and increased under drought stress conditions (Table 4). Moderate drought has a positive impact on the stomata number, while the number of stomata decreases under drastic drought stress conditions. The lower transpiration rate, thick cuticle and small stomatal aperture enhance drought tolerance in plants (Ullah et al., 2018b).

A suitable stress tolerance index must have a significant correlation with grain yield under drought stress conditions (Mitra, 2001). Almost all stress tolerance indices considered in this study were significantly correlated with grain yield under drought stress conditions. Likewise, most of stress tolerance indices were significantly correlated with grain yield under well-watered conditions except for stress susceptibility index and yield stability index. This is in line with results presented by Anwaar et al. (2020) which used stress susceptible index (SSI) to identify drought-tolerant genotypes in 50 bread wheat landraces evaluated under rainfed and irrigated conditions and showed that tolerant genotypes could be selected by low SSI and TOL values. Rosielle and Hamblin (1981) reported that genotypes that exhibited lower TOL values and high MPI values are more tolerant. Drought indices such as GMP, MP, HM, STI and YI had positive and significant associations with grain yield under both drought stress and well-watered conditions, making these indices the most suitable criteria to select for drought tolerance (Ballesta et al., 2019). Based on Harmonic mean (HM), compared to drought tolerant check genotype Alem-tena (G8,0.80), G6(0.87), G10(0.87), G11(1.08), G7(1.27), G13(1.43) and G9(1.52) had superior HM values. Schneider et al. (1997) reported that genotypes having high values of HM were found to be tolerant under drought stress conditions. Geometric mean productivity index (GMP) has often been used to determine the degree of tolerance under stress conditions. Genotypes with high value of GMP were considered as more desirable (Fernandez, 1992). Thus, we can screen durum wheat genotypes using this index and genotypes G10(0.87), G6(0.92), G14(1.04), G11(1.30), G7(1.32), G13(1.47), and G9(1.60) had high values of GMP compared to drought tolerant check (G8, 0.85). Fisher and Maurer (1978) stated that those

genotypes which have SSI value < 1 indicate genotype to be more resistant to stress. Out of 15 genotypes evaluated using this index (i.e SSI) nine of them had SSI index values <1. These were G1(0.99), G5(0.60), G6(0.74), G7(0.68), G8(0.16), G9(0.68), G10(0.38), G13 (0.53) and G14(0.6). The listed genotypes above were found to be drought resistant according to SSI criterion. Gavuzzi et al. (1997) also used yield stability index (YSI) and yield index (YI) to distinguish stable genotypes under stress and non-stress conditions and stable genotypes are expected to have high values. Accordingly, genotypes G7 (1.82), G9 (2.25), G10 (1.41), G13(2.24) and G14(1.42) had better yield index values than drought tolerant check G8 (1.37). Principal Component Analysis (PCA) revealed that traits such as spike length, number of grains per spike, grain yield and harvest index are promising traits to differentiate the tested genotypes under drought conditions. In this study, drought had a magnificent effect on yield related traits and growth parameters, in addition to the interaction effect of moisture regimes and genotypes on some growth and yield and yield related traits.

Likewise, to the present study, a study has been conducted by Ayed et al (2021) to identify drought-tolerant durum wheat genotypes from five modern varieties and six landraces in a multi-environment trial at two sites (Kef and Siliiana, Tunisia) during three growing seasons under rainfed and irrigated conditions. Six drought tolerance indices viz. mean productivity (MP), geometric mean productivity (GMP), stress susceptibility index (SSI), tolerance index (TOL), stress tolerance index (STI), and yield stability index (YSI) were used to evaluate the genotypes. The results confirmed that number of grains per spike, spike length and grain yield were significantly reduced by drought stress. Also, stress tolerance indices were found to be robust and could be used to screen genotypes for drought tolerance. Similarly, among the drought tolerance indices, GMP, MP, HM, STI and YI were found to be the most suitable for predicting drought tolerance because they had significant and positive correlations with yield under drought stress and non-stress conditions (Semahegn et al., 2020).

Materials and Methods

Description of experimental area

The study was carried out at, Hawassa University, College of Agriculture, Ethiopia under greenhouse conditions, during February to June, 2023. Hawassa is located at 7° 04"N and 38° 31" E on the escarpment of the Great Rift Valley with an average elevation of 1700 m a.s.l.

Plant materials

Fifteen durum wheat genotypes sourced from Debreziet Agricultural Research Center, Durum Wheat Breeding Program considered for this drought screening experiment and the list of these genotypes including one drought tolerant check viz. 'Alem-Tena' along with their pedigree, year of release and country of origin is depicted in the table as follows.

Table 8. List of durum wheat genotypes which were used in the experiment.

S.N	Genotype name	Code	Pedigree	Origin	Year of release
1	Kilinto	G1	DZ 918	Ethiopia	1994
2	Fetan	G2	Tob 2	CIMMYT/Ethiopia	2018
3	Selam	G3	61-130/Lds//Gll 's'/3/Cit 's'/4/Hora/3/Megrbcce 's'	Ethiopia	2004
4	Metaiya	G4	Yemen/Cit 's'//plc 's'/3/Taganrog/4/Hui 's'//Cit 71/CII/5/ Shenkora 25	Ethiopia	2004
5	Bakelcha	G5	GEDIFRA/GWEROU	ICARDA/Ethiopia	2005
6	Denbi	G6	AJAIA-/2/F3 LOCAL (SEL...ETHIO.135-85)/3/PLATA-/3//3/	CIMMYT/Ethiopia	2009
7	Bichena	G7	DZ393-4 (Illumilo/ cocorit 71 ,DZ 393-2)	Ethiopia	1995
8	Alem-tena†	G8	-	-	2017
9	Hitosa	G9	CHEN/ALTAR84/4/SRN//HU//YAV79/3/S KARV/5/LICAN/6/-9/RASCON-37	CIMMYT/Ethiopia	2009
10	Arsi-robe	G10	TOB 66	CIMMYT/Ethiopia	1996
11	Ejersa	G11	LABUND/NIGRIS 3//GAN CD98206	CIMMYT/Ethiopia	2005
12	Boohai	G12	Coo's Cndeal II, CD 3862	Ethiopia	1982
13	Flakit	G13	EN-25	CIMMYT/Ethiopia	2007
14	Mangudo	G14	ICAJIHAN 22	Ethiopia	2012
15	Malefia	G15	ALTAR84/STN..	ICARDA/Ethiopia	2005

Soil sampling, preparation and analysis

The soil for the experiment was brought from the Ethiopian wheat belt area, Arsi Negele. Soil was characterized based on soil samples taken in the field. Two composite soil samples, each made from twelve sub-samples, one for moisture content determination at field capacity and the other for the rest soil parameters determination were collected diagonally in a zigzag pattern from 0-30 cm soil depth from farmer’s field with the help of spiral auger. The composite soil sample was air dried, ground to pass through a 2 mm sieve, except for analysis of organic carbon and total nitrogen, where the sample was passed through 0.5mm sieve and stored in polythene bag for physical and chemical analysis using standard laboratory procedures. The particle size analysis was done by using the hydrometer method as outlined by FAO (2008). The pH of soil was measured in the supernatant suspension of 1:2.5 (weight/volume) soil samples to CaCl₂ solution ratio using digital pH meter (Pageet al., 1982). Soil organic carbon was determined by using the method of Walkley and Black (1934), whilst total soil nitrogen was determined using the Kjeldahl method (Dewis and Freitas, 1975).

Physico-chemical property of the experimental soil

The soil sample analysis revealed the moderately acidic nature of the soil according to Jones (2003), and total nitrogen content was very high based on Tekalign (1991) rating. Moreover, organic carbon content of the soil was medium (London, 1991) as described in Table 9. The particle size distribution showed the soil to be sandy-clay loam which is classified under textural class suitable for agricultural practices. The bulk density of the soil was 0.845 g/cm³ (Table 9). Volumetric moisture content of the soil at field capacity was 36.5%, which was used as a basis for designing the soil moisture regimes in this study (Table 9).

Table 9. Physico-chemical characteristics of the soil used in the experiment.

Physical property	Values	Chemical property	Values
Clay (%)	28.3	pH	5.64
Silt (%)	22.7	Total nitrogen (%)	0.4062
Sand (%)	49.0	Organic carbon (%)	4.7125
Textural class	Sandy clay loam	Organic matter (%)	8.1244
Bulk density (g/cm ³)	0.845		
Moisture content at FC (v/v, %)	36.48		

Greenhouse climate condition

Air temperature and relative humidity was measured for 23 days at vegetative stage by sensors located in the experimental tent to establish daily maximum and minimum temperature, relative humidity and the vapor pressure deficit (VPD). The climate data was collected by the mini data logger (ModelTesto174, Version 5.0. 2564.18771, Lenzkirch, Germany). The data logger was placed inside an open bucket to avoid direct sun and hung close to the plant canopy.

Air temperature and relative humidity data are shown in Table 10. According to the data obtained from the logger, the average maximum temperature was 33 °C while average minimum temperature was 14°C (Table 10). Regarding relative humidity, average maximum relative humidity was 94.1%, whereas average minimum relative humidity was 30.5% (Table 10). VPD computed from mean temperature and mean relative humidity was 0.71, which was low implying that there was low evaporative demand during the crop growing season, particularly at vegetative stage (Table 10).

Table 10. Greenhouse climate information.

Climatic variables	Minimum	Maximum	Mean value
Temperature (°C)	14.00	33.00	21.12
Relative humidity (%)	30.50	94.10	71.61
Vapor pressure deficit (kPa)	1.11	0.30	0.71

Note: Vapor pressure deficit (VPD) was calculated using VPD-Autogrow software; www.autogrow.com/wpcontent/uploads/2016/03/VPD_HDCALC.xls.

Determination of moisture regimes

Moisture content of the soil at field capacity

The core samples we collected, mixed together, air dried, grinded and then sieved with 2mm size sieve and then was analysed. For soil field capacity (FC) analysis soil was filled on a sample retaining rubber ring on to the 1/3 bar (-33 kpa) ceramic plate and moistened by the distilled water until its saturation. The sample with 1/3 bar ceramic plate was put in to pressure plate apparatus for 72 hours (MODEL 1500F2, Soil moisture equipment corp., SANTA BARBARA, CA., USA). After 72 hours of pressure exposure, the sample was removed from the pressure plate apparatus and weighed together with aluminum foil with sensitive balance. The weighed sample was oven dried at 105°C for 24 hours and reweighed. Eventually, the moisture content at field capacity on weight basis was determined gravimetrically and converted in to volumetric moisture content using the following relationship (FAO, 1998):

$$\theta_{FC} = \frac{SFw - SDw}{SDw} \times \left(\frac{b}{w}\right) \dots\dots\dots (1)$$

$$= 0.4319 \times \frac{0.845 \text{ g/cm}^3}{1.0 \text{ g/cm}^3}$$

= 0.3648 or 36.48%

Where, θ_{FC} = volumetric moisture content at field capacity (v/v)

SF_w = weight of wet soil (g), and

SD_w = weight of oven dry soil (g)

b = bulk density of the soil, (g/cm³)

w = density of the water 1 g/cm³

Moisture regimes

The volume of water applied to a given area would require multiplication of the depth by the area, measured in the same length units. Thus, the amounts of water to be applied per pot were determined as follows;

$$\begin{aligned} \text{Full irrigation/FI (100\% of FC)} &= \theta_{FC} \times \text{Pot depth (cm)} \times \text{Pot area (cm}^2\text{)} \dots\dots\dots (2) \\ &= 1000 \times 0.3648 \times 0.15\text{m} \\ &= 54.72 \text{ mm or } 5.472 \text{ cm} \end{aligned}$$

$$\begin{aligned} \text{100\% of FC} &= 5.472 \text{ cm} \times 190.46 \text{ cm}^2 \dots\dots\dots (3) \\ &= 1,042.197 \text{ cm}^3 \text{ or } 1.04 \text{ Litters} \end{aligned}$$

And then based on this FC value, the moisture regimes for well-watered and drought stress treatments were computed as follows:

$$\begin{aligned} 80\% \text{ of FC} &= 1.04 \text{ Litters} \times 0.8 \dots\dots\dots (4) \\ &= 0.834 \text{ Litters or } 834\text{ml} \end{aligned}$$

$$\begin{aligned} 30\% \text{ of FC} &= 1.04 \text{ Litters} \times 0.3 \dots\dots\dots (5) \\ &= 0.313 \text{ Litters or } 313\text{ml} \end{aligned}$$

The drought stress treatment was commenced at stem elongation stage (BBCH 31) and continued until physiological maturity (BBCH 87). However, until stem elongation stage all experimental units were irrigated uniformly at 80% of FC (Uwe et al., 2001).

Experimental design, treatments and experimental procedures

The experiment was laid out in a factorial completely randomized design (CRD) with two moisture regimes based on field capacity (FC) of the soil viz. well-watered (which was maintained at 80% of FC) and drought stress (maintained at 30% of FC, commencing from stem elongation (BBCH 31) till physiological maturity¹ (BBCH 87) (Table S4), two sowing densities; 5 plants per pot and 50% more and 15 durum wheat genotypes with six replicates (2×2×15×6). Hence, the experiment consisted of 60 treatment combinations, so there were 360 experimental units in total.

The soil was air dried and filled in the experimental pots of 3 liters volume, having a 17 cm top diameter, 14 cm bottom diameter and 15 cm depth at the rate of 1.79 kg/pot which was calculated on the basis of bulk density of the soil. Sowing was done on February 22, 2023. Ten seeds were sown per pot and seedlings were thinned to five and eight seedlings per pot at two leaf growth stages according to the treatment. The soil was fertilized with 173 mg a combination of blended nitrogen, phosphorus, sulfur, boron fertilizer (NPSB) and 142 mg of Urea kg⁻¹ of soil. As to the application time the whole NPSB was applied at sowing while N obtained from Urea was applied in split with 1/3 at sowing and the remaining 2/3 at tillering stage. All the experimental units were irrigated at 80% of FC until the commencement of the drought stress treatment. Pots were weighed every second irrigation.

Traits measured

The leaf area per plant, specific leaf area, leaf area ratio, stomata number, number of ear per plant, spike length, number of grains per spike, biomass yield, grain yield and harvest index were collected. In addition to these growth traits, yield and yield related attributes, and stomata number, stress tolerance indices were computed (Supplementary data).

Data analysis

Linear Mixed Model procedures were pursued considering genotypes and moisture regimes as fixed effects, while sowing density was regarded as random effect. Data were subjected to analysis using statistical software SAS version 9.4 (SAS Institute, 2016). Mean separation was done using Tukey's honestly significant difference at 5% level of significance. When there was a statistically significant interaction between the factors, the interaction was considered, rather than the main effects. Otherwise, only the main effects of treatments were presented. Graphs were plotted using Sigma Plot software version 10. Sowing density itself and the interaction of the factor with sowing density were random; these effects were not presented in the results.

Pearson's correlations were also computed using SAS software to know the magnitude and direction of correlation of parameters under well-watered and drought stress conditions.

Principal component analysis is a form of multivariate analysis utilized to reflect the importance of the largest contributor to the total variation at each axis of differentiation. In PCA, the eigenvalues are often used to determine how many factors to retain. Thus, according to Gutten's lower bound principle, eigenvalues < 1 should not be considered (Kumar et al., 2011;

¹ The BBCH scale is a standardized German coding system used to describe the growth stages of mono- and dicotyledonous plants

Jackson, 1991). Furthermore, there was no guideline to determine the significance of eigenvectors (Duzyaman, 2005). However, the coefficients (eigenvector loading) with larger absolute value for characters substantiated the relatedness of those characters with respective principal component axes (Broschat, 1979). Therefore, PCA was performed in order to select characters that contribute considerable variation to the total variation with respect to well-watered and drought stress conditions. Before analysis, the mean data of 15 durum wheat genotypes for each of the traits were first pre-standardized to mean zero and variance unity to avoid bias due to differences in measurement scales. The analysis was conducted using SAS software.

Conclusion

We found that drought had significantly affected most of the parameters studied, including stomata number. Variability in grain yield across different moisture regimes was observed, but the response patterns remained the same across genotypes particularly under drought stress conditions for plant height, number of grains per spike and grain yield. Hence, it will be worthwhile to use combinations of traits to screen and advance genotypes for successive study. Based on mean value calculation of stress tolerance indices, Pearson's correlation and Principal component analysis stress tolerance indices such as MPI, HM, GMP, STI and YI found to be robust criteria for differentiating genotypes according to their response to drought stress conditions. Under drought stress conditions, SL, NGPS, GY and HI had higher eigenvector loadings, implying that these traits could be used in screening durum wheat genotypes under moisture limited conditions. Therefore, based on preliminary evaluations, the six best thriving genotypes viz. G6 (Denbi), G7 (Bichena), G8 (Alem-tena), G9 (Hitosa), G10 (Arsi-robe) and G13 (Flakit) can be put in pipeline for release or advanced for further testing. The study was limited in not including a large number of lines, focusing instead on released varieties of durum wheat under different moisture regimes. Therefore, conducting broader screening that includes lines would be an important direction for future research. Furthermore, screening durum wheat varieties using eco-physiological and molecular traits would be an ideal strategy for more comprehensive evaluation.

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