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Assessment of Genetic Variability and Acid Soil Tolerance in Ethiopian Barley Landraces

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Abstract

Barley is an important food security and industrial crop in Ethiopia and its production is constrained by several factors including soil acidity stress. Thus, an experiment was conducted using 320 barley genotypes in alpha lattice design with two replications at Holeta, Jeldu and Midakegn testing sites during 2017 and 2018 to assess genetic variability among barley genotypes, to identify barley genotypes tolerant to acid soils using stress indices and to assess the association among stress indices as well as grain yield. Data analysis showed significant ($P \leq 0.01$) differences between the genotypes and the interactions. Estimates of heritability and genetic advance of the studied traits also revealed potential prospects for genetic improvement of traits of interest. Moreover, the overall mean grain yield under non-stress was 3212.42 kg ha^{-1} (1797 to 5936 kg ha^{-1}) compared to 2347.83 kg ha^{-1} (1797 to 5936 kg ha^{-1}) under acid soil stress indicating a yield reduction of 26.92%. Assessment of acid soil stress indices was also found to be promising in identifying tolerant genotypes with good yield potential. Yield under stress showed a strong positive correlation ($r = 0.89^{**}$) with yield under non-stress indicating that some genotypes which performed well under non-stress also showed good performance under acid soil stress. Moreover, STI, GMP, MP, AAI and ATA revealed the existence of a strong positive correlation between themselves and yield performance under both sets. Therefore, high yielding and tolerant barley genotypes were identified for further adaptation studies and simultaneous breeding line identification for subsequent crossing and variety development.

Keywords: Acid soil; Barley accession; Stress indices; *Hordeum vulgare*

Introduction

Barley (*Hordeum vulgare* L.) is the most important staple crop with an area coverage of close to one million hectares and a total annual grain production of about 2.34 million tons in Ethiopia (CSA, 2021). It is grown by smallholder farmers in Oromia, Amhara, Tigray and part of South Nations Nationalities and Peoples (SNNP) regional states with an altitude ranging from 1400 to 4000 meters above sea level (masl). Compared to malt barley production the share of food barley production is more than eighty percent in Ethiopia. Thus, barley grain accounts for more than half of the food requirement in the highland of the country for which it serves as the main source of calories and the food value of barley as source of energy is highly recognized by the farmers in Ethiopia (Ceccarelli *et al.*, 1999; Zemede, 2000). Besides the grain value of barley, its straw and grain by-

products from breweries constitute an indispensable component of animal feed in the highland where feed shortage is prevalent (Aemiro *et al.,* 2011).

Generally, barley production is hampered by several biotic and abiotic constraints among which soil acidity is now a serious threat in most central, western and southwestern highlands of Ethiopia where barley production is the most important (Getachew *et al.,* 2017). The dominant agricultural areas of the highlands which is characterized by high rainfall distribution with an altitude greater than 1500 masl are located in almost all regions of Ethiopia are affected by soil acidity (Getachew *et al.,* 2019; Hailu and Getachew, 2011). In these highland areas, crop cultivation has occurred for many years with continued removal of reserve nutrients in the harvested products. The lack of proper cultural practices which cause nutrient loss through erosion and leaching has also aggravated the problem. Moreover, it is estimated that more than 40% of the total arable land of the country has soil acidity problems (Mesfin, 2007). About 28.1% of areas are affected by soil acidity (Fig. 2) and these soils are dominated by strongly acidic to moderately acidic soils (Ermias *et al.,* 2013; Hirpa *et al.,* 2013; ATA, 2014). Under acid soil stress conditions plant growth inhibition may result from a combination of factors including Aluminum (Al), Manganese (Mn), H-ion toxicities and deficiency of essential elements (Bona *et al.,* 1993). Al toxicity is the primary limitation to agricultural production in acid soils affected areas (Rao *et al.,* 1993) and at low pH value (pH < 5.0), Al is solubilized into toxic ionic forms (Al^{3+}) which can rapidly inhibit root growth, affecting nutrient uptake, and ultimately reducing productivity (Chuan *et al.,* 1996; Soto-Cerda *et al.,* 2013). Among cereal crop species, barley is regarded as the most sensitive crop to soil acidity (Bona, 1993; Wang *et al.,* 2006) and substantial barley yield reduction due to soil acidity was also reported by various researchers in Ethiopia (Hailu and Getachew, 2011; Getachew *et al.,* 2017; Getachew *et al.,* 2019).

From a plant breeding perspective, the extension of yield potential and reduction of susceptibility to abiotic and biotic stresses are genetic forces as a foundation for crop improvement (Garvin and Carver, 2003). Generally, the basic intention of germplasm resource programs is to assure the continued availability of genetically diverse genotypes with the traits required for developing stable and productive varieties with desirable quality standards (Bockelman and Valkoun, 2011; Brown *et al.,* 2014; Tandzi *et al.,* 2019). Accordingly, the country has wealth of genetic resources of more than fifteen thousand barley accessions which were collected across the country and preserved *ex-situ* and *in-situ* by the Ethiopian Biodiversity Institute (Adugna, 2011; Bockelman and Valkoun, 2011). Ethiopian barley landraces were known for great gene diversity especially in harboring resistance genes (Qualset, 1975; Yitbarek *et al.,* 1998) and exploited worldwide by modern plant breeding endeavors (Firdissa and Heinrich, 2009).

Genetic variation allows different plant species and different varieties of the same species, to exhibit differing abilities to grow in acidic soils (Garvin and Carver, 2003). As an intervention strategy, genetic improvement is the best solution for developing barley varieties that are tolerant to soil acidity/aluminum toxicity. Studies revealed that a range of soil acidity tolerance have been identified and selective barley breeding programs have produced varieties with increased aluminum tolerance (Miao *et al.,* 2013). Hence, utilization of the conserved crop germplasm resource to develop acid soil tolerant varieties is an economically feasible and environmentally friendly management option that can complement other non-genetic approaches under acid soil environments (Getachew *et al.,* 2019; Tandzi *et al.,* 2019).

Genotype evaluation for acid soil tolerance assessment in Ethiopia is limited except for the studies in tef which were conducted by Ermias *et al.* (2013) and Misgana *et al.* (2019). In this respect, stress indices are the most useful tools for the evaluation of plant response under stress as they are the reflector of crop plant behavior under stress by relating yield under non-stress and stress conditions (Jamshid and Javnmard, 2018). Generally, several yield-based stress indices have been widely used to identify acid soil stress-tolerant genotypes based on yield loss under stress versus normal conditions (Kasno *et al.,* 2013; Dewi-Hayati *et al.,* 2014; Tandzi *et al.,* 2015). Acid soil or Aluminum Tolerance Index (ATI) and Aluminum Adaptation Index (AAI) according to Howeler (1991); Stress Tolerance Index (STI) and Geometric Mean Index (GMP) as per Fernandez (1992); Stress Susceptibility Index (SSI) according to Fischer and Maurer (1978) and Mean Productivity (MP) and Tolerance Index (TOL) according to Rosielle and Hambling (1981) are among the most utilized stress indices. Although these stress indices have been used for the evaluation of various crops under stress environments, there is limited information regarding barley genotypes assessment under soil acidity stress conditions in Ethiopia. Accordingly, a field screening experiment was executed to generate data and evaluate barley genotypes for acid soil stress tolerance. Therefore, this study was initiated to assess genetic variability among barley genotypes under acid soil stress and non-stress conditions identify barley genotypes tolerant to acid soils using stress indices and evaluate the association among stress indices as well as grain yield.

Materials and Methods

The experiments were conducted using 320 barley genotypes comprising 294 accessions obtained from the Ethiopian Biodiversity Institute (EBI, http:// www.ebi.gov.et) and twenty-six nationally released barley varieties. Among the prominent released varieties which were known for their wide adaptation and good yield potential are; HB-1307, HB-1966, IAR/H/485, Ardu-1260B, Shege,

EH-1493. Representative barley accessions of national collection across the country with full passport data were identified and purified or homogenized at Holeta Research Center. The accessions were collected from acid soil affected areas of the country were represented by; Agaw Awi and East Gojam zone in Amahara; East Shewa, North Shewa and East Wollega in Oromia; Gurage, Hadiya and North Omo in SNNP regional states. Collection areas of these barley accessions called hereafter "genotypes" for experimental purposes are described in Fig. 1. The genotypes considered were from the collections made in 16 administrative zones in the four regional states of Ethiopia (Amhara, Oromia, Tigray and SNNP). Likewise, germplasm collection points across the country are indicated on the Ethiopian map with triangular symbols marked with blue color (Fig. 1).

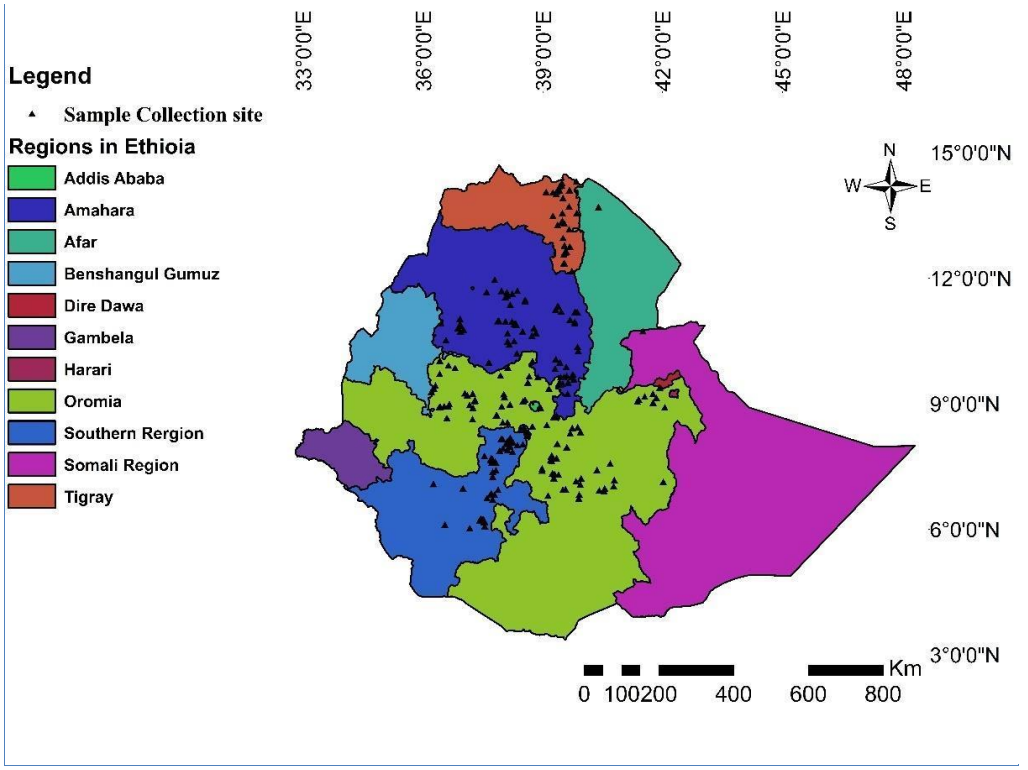


Figure 1. Map of Ethiopia showing the approximate areas of origin of barley accession and inter-regional boundaries, whereas different colors designate regional states of the country. The triangular points were developed from the geographic coordinate (latitude and longitude) position of barley collection points.

Test Environment and Experimental Design

Based on various reports from the soil research team at Holeta Agricultural Research Center, soil samples were collected and analyzed at the Holeta soil laboratory to determine the appropriate acid soil test environment. Accordingly, soil parameters such as pH (1:1.25 H_2O) exchangeable acidity and exchangeable

aluminum were quantified. Based on the result of soil samples, experimental fields were identified at Holeta, Jeldu and Midakegn sites depending on the level of soil acidity. Description of the study locations for geographical position and soil physico-chemical properties are shown in Table 1.

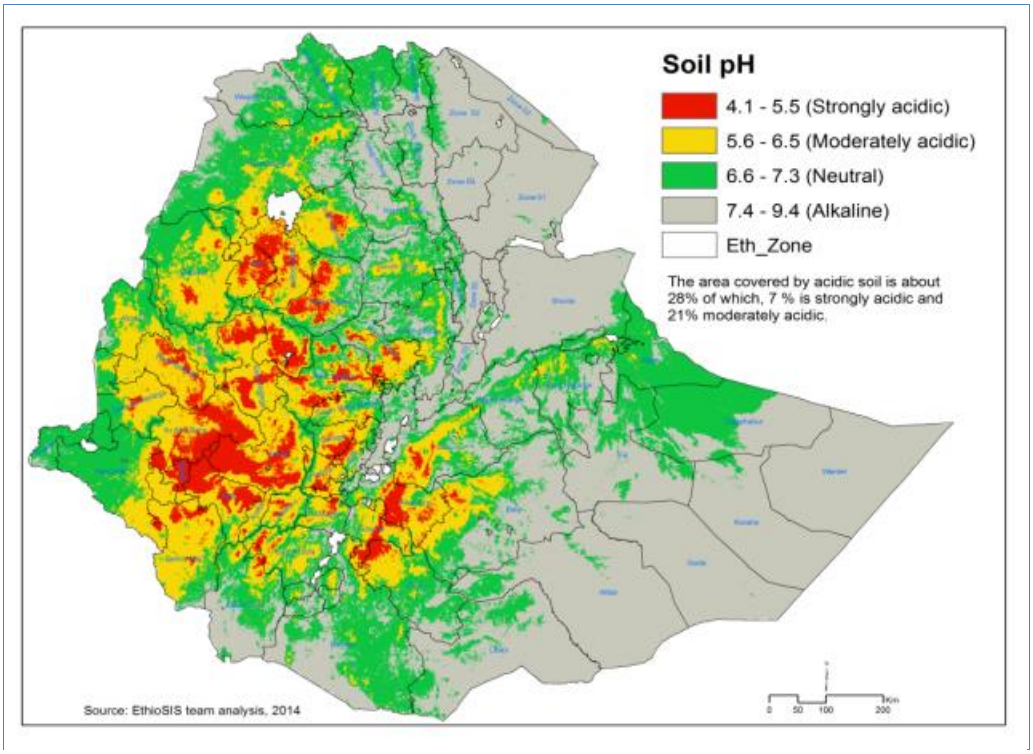


Figure 2. Extent and distribution of soil acidity in Ethiopia; (adopted from ATA, 2014).

Accordingly, the selected experimental field was divided into two equal parts side by side at the respective site. Then the fields were assigned to lime treated (non-stress) and without lime treatment (acid soil stress) experimental sets respectively. In stress breeding, researchers recommend that genotype evaluation and selection needs to be done under both stress and non-stress conditions (Fischer and Maurer, 1978; Fernandez, 1992). For the non-stress field, $CaCO_3$ or lime requirement was determined based on exchangeable acidity which was estimated through extraction and titration as described by Kamprath (1984).

$$LR\left(CaCO_3(\,kg/ha)\right) = \frac{EA\left(\frac{cmol}{kgsoil}\right) \times DS\,(m) \times A\,(m^2) \times \rho_b\left(\frac{g}{cm^3}\right)}{2} \times LF$$

Where: LR= Lime Rate; EA= Exchangeable Acidity; DS= Depth of Soil (0.15m); A= Area of experimental land; ρb= Soil bulk density; LF= Liming Factor or adjustment factor (LF= 1.5) is determined based on crop response.

Fifteen cm plow depth, bulk density of the soil and area (m^2) of the experimental field were used for lime rate (LR) determination. Subsequently, the required amount of fine lime was incorporated into the soil thirty days before planting to get sufficient incubation time.

Table 1. Description of the test locations for geographical position and physico-chemical properties of the soils.

SN	Experimental Site	pH	Ex A	ExAl	*ρd*	Altitude	Longitude	Latitude
1	Holeta Research Center	4.86	1.55	1.05	1.13	2400	$38^0$30'17E	$9^0$03'28N
2	Jeldu (Kolu)	4.08	2.58	1.74	1.26	2800	$38^0$03'54E	$9^0$17'50N
3	Midakegn (Baro Bidaru)	4.07	3.74	2.62	1.15	2900	$37^0$28'25E	$9^0$08'35N

Ex A= Exchangeable acidity; Ex Al= exchangeable aluminum; b= Soil bulk density;

Accordingly, each set of experiments was conducted separately under acidic soil and lime treated optimum conditions at three locations for two consecutive years. The experiments were conducted in an alpha lattice design replicated twice, with 20 incomplete blocks, each containing 16 genotypes. Plot area of 2.0 m^2 consisting of four rows 2.5 m long spaced 0.2 m apart between rows, 0.4 m between plots and 1.5 m between blocks was considered. Seeds were sown on rows with manual drilling at a rate of 85 kg ha^{-1}. Likewise, fertilizers were applied at the rate of 46 kg ha^{-1} P_2O_5 and 41 kg ha^{-1} Nitrogen from NPS (Nitrogen, Phosphorus, Sulphur) formulation and Urea source respectively once at planting based on the research recommendation in the study area. The ratio of N:P:S is 19: 38: 7 for Nitrogen, P_2O_5 and Sulphure respectively. Generally, both experimental sets received similar management except for lime treatment.

Data Collection

Data were collected at all locations from both sets of experiments either from the whole plot or from ten randomly sampled plant bases in each plot. Mean values of the 10 randomly sampled plants and plot basis were used to estimate the performance of each genotype for the traits considered (Table 2).

Table 2. Descriptions of morpho-agronomic traits of barley genotypes on plot and plant basis

Traits	Description of data collection
Days for Heading	Recorded as the number of days from sowing to the date on which 50% of the plants in a plot have produced their first flower.
Days to Maturity	Recorded as the number of days from sowing to the stage when 75% of the plants in a plot have reached maturity.
Grain Filling Period	Number of days between days to flowering and days to physiological maturity
1000-Kernel Weight	Weight in grams of random samples of thousand kernels per plot.
Hectoliter Weight	Hectoliter weight (kg/hl) is flour density produced in a hectoliter of the seed and it was determined using moisture and hectoliter analyzer.
Biological Yield	Biomass yield was determined by weighing the total air-dried above-ground biomass harvested from the plot and expressed in kg ha^{-1}.
Grain Yield	Grain yield was determined by weighing grain samples from a plot adjusted to 12% moisture content and expressed in kg ha^{-1}. Grain yield adjustment or correction factor; CF= (100-Actulal Moisture 100-Standrd Moisture)
Stress Score	Acid soil stress score was recorded based on (1-9) scale, in which 1 is when there is very low sign of stress and 9 is high stress susceptibility (IPGRI, 1994).
Plant Height	Measured as a height in cm from the soil surface to the tip of the spike excluding the awns at maturity and expressed as an average of ten plants.
Fertile tillers /Plant	Number of fertile tillers per plant excluding the main plant was recorded at maturity and expressed as an average of ten plants in a plot.
Spike Length	Spike length of main tiller measured in cm from base to tip excluding the awns and expressed as an average of ten plants in a plot.
Kernel Number Per Spike	Determined by counting the number of kernels produced on the main tiller of each plant and expressed as an average of ten plants in a plot.
Kernel Weight Per Spike	Determined by weighing the kernels in grams in each spike of the main tiller of each plant and expressed as an average of ten plants in a plot.
Spike Weight	Determined by weighing the spike of the main plant as an average of ten plants in plot.

Statistical Analysis of Phenotypic Traits

All quantitative traits data were subjected to analysis of variance (ANOVA) using META-R version 6.0 developed by CIMMYT (Alvarado *et al.*, 2016; Alvarado *et al.*, 2020) and Minitab software version 17 statistical software packages (Minitab, 2007). Traits with count and scale data were log and square root transformed before analysis according to Gomez and Gomez (1984). Analysis of variance was done first separately for each environment under respective management. For the combined analysis of variance, the homogeneity of error variance was tested using the F-max method from a separate analysis, which is the ratio of the larger variance to the smaller variance (Hartley, 1950; Gomez and Gomez, 1984).

The total variability for the traits of days to heading, days to maturity, grain filling period, plant height, number of fertile tillers, spike length, spike weight, number of kernels per spike, thousand kernel weight, hectoliter weight, grain yield, and biomass yield were quantified and individual as well as combined analyses of variance over test environments using the following models:

The linear models were implemented in *lmer* from package *lme4* of R using REML to calculate BLUPs and estimate the variance components for individual and combined analysis. For individual trial; $Y_{ijk} = \mu + Rep_i + Block_j\ (Rep_i) + Gen_k + \varepsilon_{ijk}$ and for the combined analysis across all environments for the lattice design, is based on the model; $Y_{ijkl} = \mu + Env_i + Rep_j(Env_i) + Block_k(Env_i\ Rep_j) + Gen_l + Env_i \times Gen_l + \varepsilon_{ijkl;}$ where Y_{ijkl} is the trait of interest, μ is the mean effect, Rep_j is the effect of the j^{th} replicate within i^{th} environment, $Block_k$ (Rep_j) is the effect of the k^{th} incomplete block within the i^{th} environment and j^{th} replicate, Gen_l is the effect of the l^{th} genotype, and $Env_i \times Gen_l$ are the effects of the i^{th} environment and the environment × genotype interaction, ε_{ijkl} is the error associated with the i^{th} environment, j^{th} replication, k^{th} incomplete block and the l^{th} genotype, which is assumed to be normally and independently distributed, with mean zero and homocedastic variance σ^2. When using META-R for calculating the BLUPs, all effects (including environment) were considered random (Alvarado *et al.*, 2016; Alvarado *et al.*, 2020).

In the current study, seven grain yield based stress indices were used to evaluate acid soil stress tolerance and susceptible barley genotypes. Description of all the stress indices with their respective formula are shown in Table 3. Moreover, the reduction in overall trait mean values due to acid soil stress was calculated as percent mean reduction; PMR (%) = $\frac{(Yns-Yst)}{Yns} \times 100$; Where; Yns and Yst are yields of a given genotype under non-stress and stressed soil conditions respectively.

The broad-sense heritability for the combined analyses was calculated as;

$h^2 = [\frac{(\sigma^2 g)}{\sigma^2 g + \sigma^2 g\ e\ /n\ env + \sigma^2 e/\ n\ env \times n\ reps}] \times 100$; where σ^2g, σ^2ge, σ^2e, n env, and n reps are; genotype, genotype by environment interaction variance, error variance components, number of environments and number of replications respectively. Phenotype variance was computed from the summation of $\sigma^2 g + (\sigma^2 ge/n\ env) + (\sigma^2 e/\ n\ env \times n\ reps)$.

Genetic advance in an absolute unit (GA) and percent of the mean (GAM), assuming selection of the superior 5% of the genotypes, was estimated following the methods illustrated in Johnson *et al.* (1955) and Brown *et al.* (2014) as:

$GA = K \times \sigma Ph \times h^2$ $\qquad GAM = [\frac{GA}{\bar{X}}] \times 100$

Where σ_{Ph}= phenotypic standard deviation, h^2 = broad sense heritability and $\bar{X}$ = Grand mean, K= the standardized selection differential at 5% selection intensity (k = 2.063).

Pearson correlation coefficient and principal component analysis were carried out using those indices along with grain yield under stress and non-stress. The correlation coefficient is useful in finding out the overall degree of linear

association between two traits. Even, a better approach than a correlation analysis such as biplot analysis is needed to identify superior genotypes for both stress and non-stress environment for assessing relationships among all attributes at once (Talebi *et al.,* 2009; Nazari and Pakniyat, 2010; Teklay *et al.,* 2020). Principal component analyses for yield-based stress indices and grain yield was carried out using R software and the values of various indices and yield under both conditions were pre-standardized to means of zero and variances of unity before principal component analysis to avoid bias due to differences in values or measurement scales (Manly, 1986).

Table 3. Description of the selected acid soil stress indices

Tolerance Index	Formula	Reference	Remark
Stress Susceptibility Index (SSI)	$SSI = \frac{(Yns-Yst)}{(Yns*(1-[\frac{\mu Yst}{\mu Yns}]))}$	Fischer and Maurer (1978)	Values of SSI < 1 denotes high yield stability and values > 1 indicate high stress susceptibility
Tolerance Index (TOL)	$TOL = (Yns - Yst)$	Rosielle and Hambling (1981)	Highest values for TOL indicate greater yield reduction due to stress, whereas low values show tolerance
Stress Tolerance Index (STI)	$STI = \frac{(Yns)(Yst)}{(\mu Yns)^2}$	Fernandez (1992)	Highest values STI indicates stress tolerant genotype
Aluminum Adaption Index (AAI)	$AAI = \frac{(Yns)(Yst)}{(\mu Yns)(\mu Yst)}$	Howeler (1991)	Highest values of AAI designate stress tolerant genotype
Mean Productivity (MP)	$MP = \frac{(Yns+Yst)}{2}$	Rosielle and Hambling (1981)	Highest values of MP means higher stress tolerance and yield potential for genotype
Geometric Mean Index (GMP)	$GMP = \sqrt{Yns \times Yst}$	Fernandez (1992)	Highest values of GMP designate high yield potential of genotype under stress and non-stress
Yield stability Index or Aluminum Tolerance Index (ATI)	$ATI = \left(\frac{Yst}{Yns}\right)$	Bouslama and Schapaugh (1984); Howeler (1991)	Genotype with high values of ATI designate stable under stress and non-stress

Where; Yns and Yst are yields of a given genotype under non-stress and under stress soil conditions respectively. μYst is mean yield of all test genotypes under stress conditions whereas μYns is mean yield of all genotypes under non-stress soil conditions.

Result and Discussion

Analysis of Variance

Individual analysis of variance for each environment revealed significant ($P \leq 0.01$) differences among genotypes for the trait studied. Overall mean grain yield at Holeta-17, Holeta-18, Jeldu-17, Jeldu-18 and Midakegn-18 were 2387, 2568, 1195, 2092 and 839 kg ha^{-1} under stress; whereas 2777, 2897, 2835, 3870 and 3705 kg ha^{-1} under non-stress respectively. After checking for homogeneity of variance combined analysis of variance revealed highly significant ($P \leq 0.01$) differences among the genotypes under both trials sets indicating adequate

variability for the trait studied. Likewise, environment and genotypes by environment interaction also showed significant differences for most of the characters except biomass yield, spike weight, number of kernels per spike and plant height. Combined analysis of variance for the lime treated experiments at Holeta, Jeldu and Midakegn locations over two years showed highly significant ($P \leq 0.01$) differences among genotypes for grain yield, biological yield, days to heading and maturity, grain filling period, hectoliter weight, thousand kernel weight, number of fertile tillers, spike length, plant height, spike weight, number of kernels per spike and kernel weight per spike (Table 4a).

Similarly, the result of the experiment under acid soil stress is presented in Table 4b. In this experiment combined analysis was based on three environments excluding Jeldu-17 and Midakegn-18 owing to variance heterogeneity and transformation couldn't stabilize the variance heterogeneity. Analysis of variance for the experiments combined over Holeta-17, Holeta-18 and Jeldu-18 showed significant ($P \leq 0.01$) differences among genotypes and genotype by environment interaction for most of the morpho-agronomic traits studied. Moreover, the heritability of the traits ranging from 58% to 97% and 58% to 94%; genetic advance from 6.3% to 67.3% and 7.04% to 76.8% under non-stress and stress conditions respectively, indicating potential prospect for genetic improvement in traits of interest (Table 3a and 3b). Heritability of all traits showed a similar trend under both sets of experiments though relatively low under acid stress compared to non-stress conditions.

Table 4a. Variance components for some agronomic traits of barley genotypes under lime treated environments at Holeta, Jeldu and Midakegn testing locations.

Statistics	[1]DTH	DTM	GFP	PHT	FT	SPL	HLW	TKW	GY	BMY	SPW	NKPS	SWPS
Genotype	46.02**	70.02**	5.06**	65.89**	0.12**	0.655**	5.50**	26.77**	762671.66**	6814001.11**	0.387**	140.11**	0.25**
Env	11.46**	401.41**	255.2**	62.88**	1.48**	0.187ns	5.05**	33.21**	173480.58ns	0.0001ns	0.0001ns	1.08ns	0.004ns
G x E	3.52**	9.67**	7.88**	12.36**	0.066**	0.091**	4.19**	5.41**	421435.21**	1669250.65**	0.001ns	4.79**	0.012**
Residual	6.488	16.535	20.288	33.985	0.392	0.528	14.81	6.771	586011.94	3692073.94	0.118	26.503	0.094
Mean	64.48	119.40	56.69	103.31	2.96	6.97	63.70	44.51	3212.42	8748.77	2.11	35.65	1.792
CV (%)	3.95	3.41	7.95	5.64	21.14	10.43	6.04	5.85	23.83	21.96	16.31	14.44	17.086
h^2	0.96	0.94	0.58	0.92	0.69	0.90	0.65	0.94	0.84	0.91	0.93	0.97	0.927
GA (%)	21.28	13.99	6.25	15.51	20.0	22.74	6.05	23.19	51.36	58.52	58.53	67.33	54.91

Note: **, * Significant difference at (P ≤ 0.01), (P≤ 0.05) respectively and ns: non significant difference.

Table 4b. Variance components for agronomic traits of barley genotypes under acidic soil environments at Holeta, and Jeldu locations.

Statistics	[1]DTH	DTM	GFP	PHT	FT	SPL	HLW	TKW	GY	BMY	SPW	NKPS	SWPS
Genotype	50.475**	90.80**	13.95**	71.76**	0.136**	0.75**	7.62**	25.01**	860231.78**	8476170.42**	0.222**	110.38**	0.166**
Env	14.88*	222.47**	129.62**	38.77ns	0.857*	0.696*	5.60**	26.29**	0.001ns	1790928.82ns	0.0001ns	8.14ns	0.001ns
G x E	5.62**	6.39**	3.24**	4.81ns	0.075**	0.026**	6.96**	5.85**	293443.61**	0.0001ns	0.056**	7.81**	0.05**
Residual	8.281	29.72	31.25	78.911	0.432	0.725	11.37	15.381	714631.52	5431824.92	0.187	37.854	0.148
Mean	66.15	120.09	53.95	93.84	2.17	6.80	61.69	41.26	2347.83	7420.54	1.77	32.13	1.54
CV (%)	4.35	4.54	10.36	9.47	30.27	12.52	5.47	9.50	36.00	31.41	24.32	19.15	25.08
h^2	0.94	0.93	0.69	0.83	0.58	0.85	0.64	0.85	0.80	0.90	0.75	0.93	0.73
GA (%)	21.44	15.74	11.84	16.94	26.70	24.24	7.40	22.98	72.75	76.83	47.24	64.79	46.60
PMR (%)	-2.52	-0.58	4.83	9.16	26.69	2.44	3.16	7.30	26.91	15.18	16.11	9.87	13.96

Note: **, * Significant difference at (P ≤ 0.01), (P≤ 0.05) respectively and ns: non significant difference.

[1]DTH= days to 50%heading, DTM= days to 50% maturity, GFP= number of days from heading to maturity, PHT=plant height(cm), NFT=number of fertile tillers, SPL=spike length(cm), HLW= hectoliter weight(kg/hl), TKW=thousand kernel weight(g), GY=grain yield (kg/ha), BY=biological yield(kg/ha), SPW=spike weight(g), NKPS= number of kernels per spike, KWPS=kernel weight per spike(g), PMR(%) = Percent Mean Reduction

The overall mean grain yield under non-stress was 3212.42 kg ha^{-1} compared to 2347.83 kg ha^{-1} in the stress condition, indicating a grain yield reduction of 26.92%. Moreover, the maximum and minimum yield performance under acid stress and non-stress soil conditions were 4722.8 kg ha^{-1}, 1142.8 kg ha^{-1} and 5932.8 kg ha^{-1}, 1791.5 kg ha^{-1} respectively. On the other hand, the relative overall mean performance of all traits except days to heading and days to maturity showed reduction under acid soil stress with varying magnitude in which the highest reduction was indicated in grain yield followed by fertile tillers per plant, spike weight, and biological yield (Table 4b). The test of mean comparison of the top ten percent of barley genotypes for their morpho-agronomic traits was presented in Table 6. Out of the total barley genotypes evaluated, twenty genotypes were among the top 10 percent showing high yield performance with varying magnitude under stress and non-stress soil conditions (Table 7a and Table 7b).

Generally, the current study disclosed the possibility of identifying lines with better grain yield, acid soil stress tolerance and other desirable attributes for further evaluation and subsequent breeding lines development to address the acid soil problem. According to Brown *et al.* (2014) observable phenotypic variation among or within the crop population and the requirement for phenotypic variation to have a genetic basis are the prerequisites in selective breeding. Then, selection will only be successful if there is sufficient phenotypic variation and if at least some of this variation has a genetic in origin. To this end, these barley genotypes would be valuable as a source of breeding materials for future variety improvement. Accordingly, Sintayehu and Tesfahun (2011) described genetic diversity and character association in barley genotypes evaluated in Arsi area indicated significant variation for various traits.

In a similar study conducted on genetic variability, heritability and genetic advance in seed yield and yield related traits in Ethiopian barley genotypes, Zerihun *et al.* (2011) reported significant genotypic variation in some important traits. Ceccarelli and Stefania (2000) also noted that in the barley landrace study, the heritability estimates under stress are even higher than in non-stress sites and high genetic variability is expressed within the landraces under stress. A similar observation of genetic variability was reported in the study of acid soil stress tolerance in maize (Tandzi *et al.,* 2015). In general, researchers reported varying ranges of genetic variability and heritability in several crop breeding and evaluation which is attributed to the type of genetic materials, a trait to be measured and environmental condition to which the individuals are subjected (Falconer and Mackay, 1989; Dabholkar, 1992; Ceccarelli and Grando, 1996).

Exploration of Barley Genotypes for Acid Soil Tolerance using Stress Indices

Barley productivity is hampered by several production constraints among which soil acidity is now becoming a serious threat to crop production in most central, western and southwestern highlands of Ethiopia (Getachew *et al.,* 2017; Getachew *et al.,* 2019). Preliminary observation of soil samples in the central highland of west Shewa indicates the extent of severity of soil acidity in the current scenario (Table 1). Several screening methods for acid soil tolerance have been employed from genotype screening in the laboratory to soil bioassay and field evaluation (Hede *et al.,* 2001). Field screening techniques is the most direct way of acid soil tolerance study for both grain and total biomass performance under field conditions which facilitates evaluation of large populations and allows estimation of yield under natural soil and climatic conditions in which resistant varieties are ultimately grown (Howeler, 1991; Singh, 2007). Consequently, analysis of variance for grain yield and related agronomic traits in this study revealed the presence of a considerable genotypic variation under non-stress and stress conditions (Table 4a and Table 4b) thereby suggesting the possibility of selecting better-performing genotypes.

The variation in yield reduction under low soil pH is based on the level of exchangeable acidity and exchangeable Aluminum in the soil, the agro-climatic conditions of the environment, and the genetic potential of crop genotypes (Tandzi *et al.,* 2019). As indicated in soil analysis result in Table 1, the extent of soil acidity at Jeldu and Midakegn districts was very intense which caused substantial yield reduction. Accordingly, the severity of stress in some of the environments caused varying magnitude of substantial grain yield reduction of 14%, 11%, 58%, 46% and 77% at Holeta-17, Holeta-18, Jeldu-17, Jeldu-18 and Midakegn-18 test environments respectively (data not shown).

Based on stress tolerance index (STI), stress susceptibility index (SSI) and geometric mean productivity (GMP) indices; 22% of the barley genotypes were categorized to be tolerant, as indicated with high values of STI, AAI, and GMP and low values of TOL and SSI. Accordingly, mean values of STI, AAI, GMP, TOL and SSI were 1.57, 2.15, 4008.3, 367.4 and 0.42 respectively. However, 48% of the genotypes were susceptible based on stress index characteristics of low STI, AAI and GMP, high TOL and SSI values. The mean values of these indices were 0.43, 0.59, 2098, 1169.9 and 1.41 respectively. Likewise, 30% of the evaluated barley genotypes showed STI, AAI, GMP, TOL, SSI values of 0.79, 1.08, 2837.6, 745.5, and 0.89 respectively indicating intermediate type. Some prominent improved varieties known for their good yield potential and wide adaptation were grouped under tolerant and intermediate types as elaborated in principal component analysis (Fig. 5). In the current study STI, AAI, GMP and MP were identified as good indices in identifying tolerant and high yielding genotypes in a

similar trend. Those genotypes in the top ten percent also showed top values in these indices. Moreover, SSI and TOL were similar in identifying genotypes. Thus, promising breeding lines with good acid soil stress tolerance and yield potential can be extracted from those genotypes for subsequent crossing works. According to Fernandez (1992) stress assessment based on STI and GMP helps in the selection of genotypes with higher stress tolerance and yield potential. Likewise, SSI estimates the rate of change for each genotype in yield between the stress and non-stress conditions relative to the mean change for all genotypes. Values of SSI higher than one indicate high-stress susceptibility or poor yield stability and values lower than unity denotes low susceptibility or high yield stability (Kemelow and Alemayehu, 2011; Saad *et al.,* 2014). Karami *et al*. (2005) reported that MP, GMP and STI are the most proper indices in barley for assessing tolerance to a given stress.

Correlation among Stress Indices and Grain Yield

The Pearson correlation coefficients of grain yield under a non-stress environment or limed treated (Yns) and grain yield under acid soil stress (Yst) conditions with various tolerance indices are shown in Fig. 3 and Fig. 4A, that Yns was significantly and positively correlated with Yst ($r= 0.89^{**}$), mean productivity ($r= 0.97^{**}$), geometric mean productivity ($r= 0.96^{**}$), stress tolerance index ($r= 0.96^{**}$), Acid soil or Aluminum tolerance index ($r= 0.34^{**}$), Aluminum adaptation index ($r= 0.96^{**}$) and tolerance index ($r= 0.18^{**}$), while significantly and negatively correlated with stress susceptibility index ($r= -0.34^{**}$).

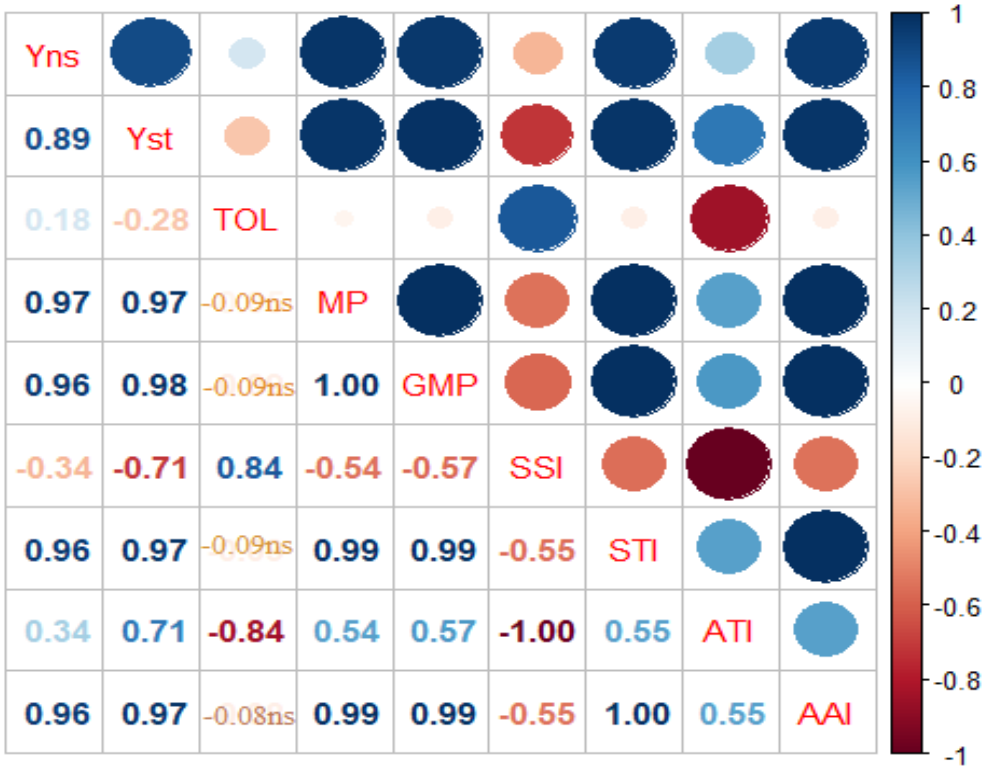
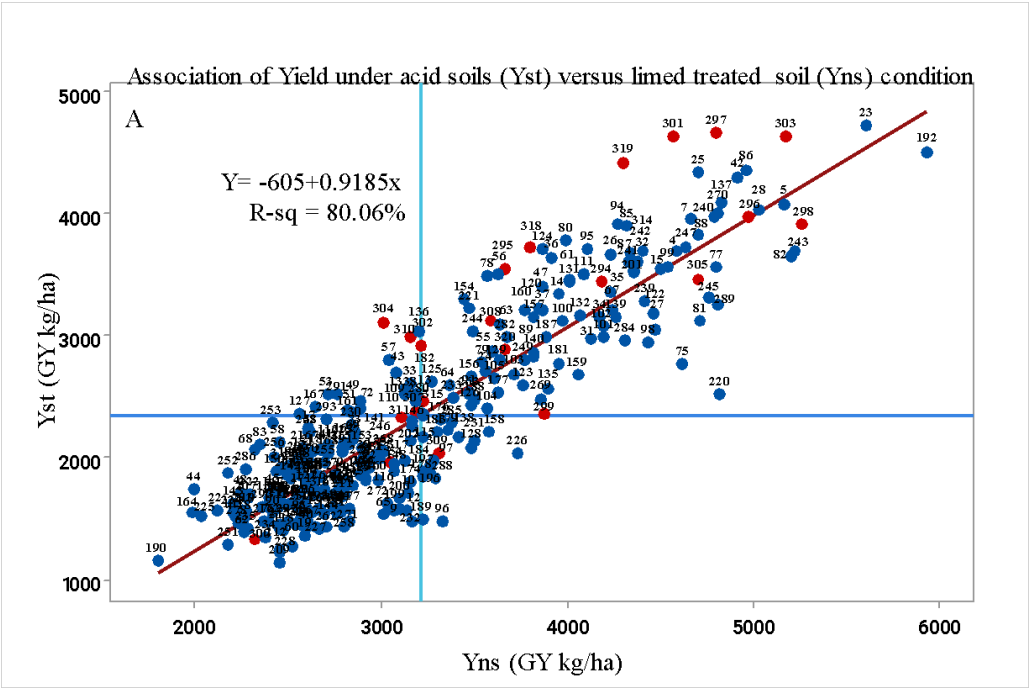


Fig 3. Heatmap of Pearson correlation coefficient matrix using grain yield and yield based stress indices of barley genotypes evaluated under acid soil stress(Yst) and non-stress(Ynst). SSI: Stress Susceptibility Index; TOL: Tolerance Index; STI: Stress Tolerance Index; AAI: Aluminum Adaption Index; MP: Mean Productivity; GMP: Geometric Mean Index; ATI: Al Tolerance Index

Similarly, grain yield in the Yst was significantly and positively correlated with mean productivity (r= 0.97**), geometric mean productivity (r= 0.98**), and stress tolerance index (r= 0.97**), Aluminum tolerance index (r= 0.71**), Aluminum adaptation index (r= 0.97**) but significantly and negatively correlated with stress susceptibility index (r= -0.71**) and (r= -0.28**) tolerance index. Previous findings reported that MP, GMP and STI are the most appropriate indices in barley, wheat, maize and sorghum for assessment of stress tolerance and high-yielding genotypes (Karami *et al.*, 2005; Nazari and Pakniyat, 2010; Drikvand *et al.*, 2012; Saad *et al.*, 2014; Tandzi *et al.*, 2015; Teklay *et al.*, 2020). Indices STI, GMP, MP, AAI and ATA showed the existence of a strong positive correlation among themselves showing similarity between these indices for genotypes ranking (Fig. 5). Teklay *et al.* (2020) also reported similar results in sorghum genotype evaluation for stress tolerance. According to Farshadfar *et al.* (2001) most suitable indices for selecting stress-tolerant cultivars is an index that has a relatively strong correlation with the grain yield under stress and non-stress conditions. On the other hand, strong correlations were found among SSI and TOL depicting that they can be used interchangeably for screening under stress

conditions (Fig. 4; Fig. 5). Likewise, the significant negative correlation of indices with yield under stress suggests that relatively low yield reduction, low-stress susceptibility index and low tolerance index values could be used to select high yielding genotypes under acidic soil environments (Golabadi and Maibody, 2006; Talebi *et al.*, 2009; Tandzi *et al.*, 2015). The correlation coefficient of the tolerance index with yield under stress was r= -0.28 which shows selection based on tolerance should decrease yield in the stress environment and increase grain yield under non-stress as indicated in r= 0.18, despite a weak positive association.



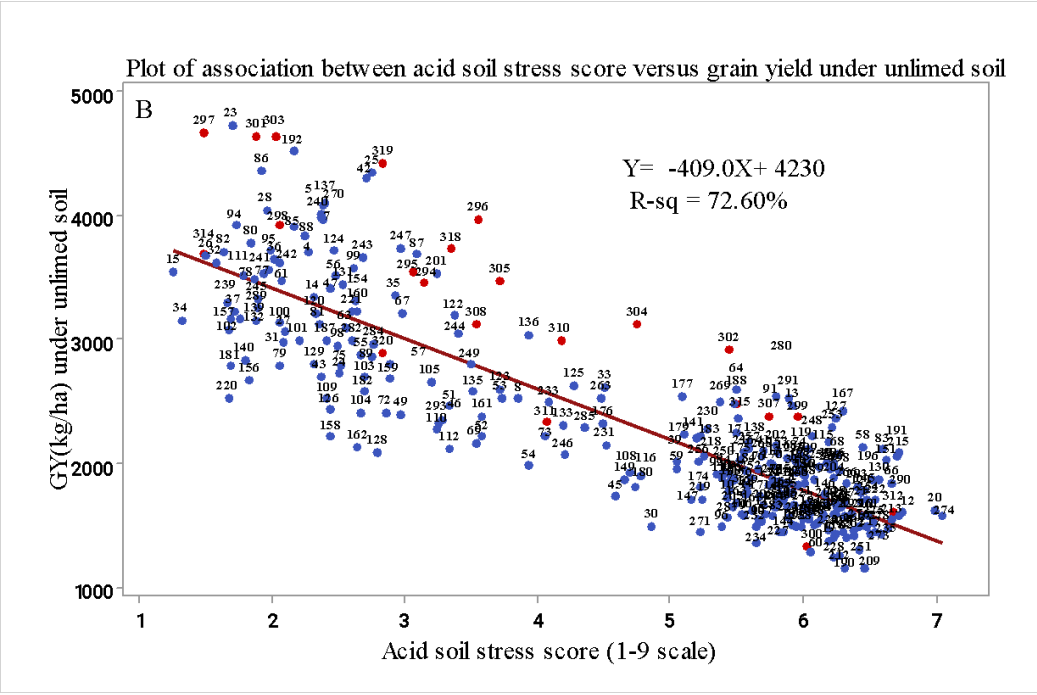


Figure 4. Interrelationship of grain yield of 320 barley genotypes grown under acid soil stress versus non-stress environments, broken line designate mean yield of respective environment (A); Grain yield under acid soil stress versus (1-9) stress scoring values (B); Improved varieties were designated with red color.

A positive correlation between TOL and Yns and a negative correlation between TOL and Yst suggested that selection based on TOL will lead to the reduction of yield under stress and an increased yield under non-stress conditions and this is in agreement with the study by Talebi *et al.* (2009) and Teklay *et al.* (2020). The negative association of SSI and TOL with grain yield under stress indicated that genotypes with low SSI and TOL values had lower yield differences between non-stress and stress environments. The association of grain yield under stress versus non-stress environments showed a significant positive ($r= 0.89^{**}$) correlation indicating that genotypes that performed well under non-stress also performed well under stress conditions. Moreover, significant negative association ($r = -0.85^{**}$) between grain yield under stress and stress score based on (1-9) scale in which score one designates stress-free whereas nine for acid soil stress susceptible genotypes (Fig. 4B). In general, this result depicts prospect of some barley genotypes as a promising germplasm source for improvement of yield potential performance and acid soil tolerant variety development.

According to Falconer (1989), consistent performance of some genotypes under contrasting environments may represent nearly the same character determined by the same set of genes. Moreover, the result of correlation analysis among various indices displayed both positive and negative associations showing that some of

the indices are generally similar but others are dissimilar in genotype ranking, respectively. Generally, the strong positive correlation between grain yield under stress and non-stress environment implied the possibility of direct selection for stress conditions based on performance under non-stress conditions (Horst, 2000; Negarestani *et al.*, 2019; Talebi *et al.*, 2009). However, Drikvand *et al.* (2012) reported a lack of association between yield under stress and non-stress environment suggesting the feasibility of an independent breeding approach.

Principal Component Analysis

The result of principal component analysis showed that the four principal components accounted for 100% of the variation. The first and second principal components accounted for 99.3% of total variability suggesting that the two principal components adequately explained the variation in the data. The first principal component (PC1) explained 74.6% with high loading due to grain yield in the stress (0.386), geometric mean productivity (0.378), stress tolerance index (0.375), mean productivity (0.375), Al tolerance index (0.280), Al adaptation index (0.375) and grain yield in the non-stress (0.343). The second principal component (PC2) also explained 24.7% of the total variation with high loading due to the tolerance index (0.634), stress susceptibility index (0.458), and grain yield under non-stress (0.305). PC1 showed a positive correlation with STI, ATI, AAI, GMP, and MP indices as well as grain yields under stress and non-stress, whereas PC2 showed a positive association with all stress indices except ATI (Table 4).

Figure 5. Biplot based on two components obtained from PCA using Yield under limed (Yns), Yield under unlimed(Yst), Tolerance index(TOL), Geometric mean productivity(GMP), Mean productivity(MP), Stress tolerance index(STI), Stress susceptibility index(SSI), Al tolerance index(ATI), Al adaptation index(AAI). Group:In (intermediate); Su (Susuptible); To (Tolerant)

Likewise, barley genotypes were subjected to biplot analysis to determine their relationship among stress indices (Fig. 5). Biplot of PC1 and PC2 for 320 barley genotypes described with genotypes number and/or varieties depicted that genotypes 222969-C(192), 16726-A(23), 24987-B(86), 17148(42), 1773-C(5), 16737-B(25), 16739-D(28), 212947-A(137), 242093-A(270), 235541-A(240), 3514-A(7), 1773-B(4), Balemi(314) and improved varieties HB-1307(303), Shege(301), Ardu12-60B(298), EH-1493(296), IAR/H/485(297), HB-1966(319) were among the genotypes located near to the stress indices (STI, ATI, AAI, GMP, GP) indicating strong association of these genotypes with the indices having high PC1 but low PC2 values. Moreover, these genotypes were characterized by high yield potential and STI values greater than 1 as well as small SSI values less than 1 which is an indication of good stress tolerance (data not shown). Golabadi and Maibody (2006), Negarestani *et al.* (2019) reported similar results of positive and strong correlations of MP, GMP and STI with Yns and Yst.

Table 5. Principal component analysis for grain yield of 320 barley genotypes under acid soil stress and non-stress conditions and stress tolerance indices.

	PC1	PC2	PC3	PC4	PC5
Indices[1]	Eigenvectors				
Grain Yield under Limed (YP)	0.343	0.305	-0.229	0.200	0.282
Grain Yield under Stress (YS)	0.386	0.010	0.040	0.371	0.280
Tolerance index (TOL)	-0.114	0.634	-0.582	-0.388	-0.012
Mean productivity (MP)	0.375	0.160	-0.095	0.295	0.289
Geometric mean productivity (GMP)	0.378	0.135	-0.090	0.271	-0.870
Stress susceptibility index (SSI)	-0.280	0.458	0.389	0.247	-0.014
Stress tolerance index (STI)	0.375	0.145	0.379	-0.441	0.009
Aluminum tolerance index (ATI)	0.280	-0.458	-0.389	-0.247	0.014
Aluminum adaptation index (AAI)	0.375	0.145	0.379	-0.441	0.009
Eigenvalue	6.713	2.224	0.053	0.009	0.0001
Variability (%)	74.6	24.7	0.6	0.1	0.000
Cumulative (%)	74.6	99.3	99.9	100.0	100.0

Table 6. Comparison of the mean performances of the top 10% genotypes with mean values of population for the studied barley genotypes under acid soil stress.

SN	Traits	Mean of selected genotypes ($\bar{X}$)	Population parameter(μ)	Change through selection ($\bar{X}$-μ)	Change as % of pop. parameter(m)	Z-test
1	DTH	78.57	66.15	12.41	18.77	10.22**
2	DTM	134.25	120.09	14.15	11.79	8.74**
3	GFP	59.50	53.96	5.53	10.25	10.245**
4	PHT	107.03	93.84	13.19	14.05	9.75**
5	FT	2.67	2.17	0.50	22.98	10.20**
6	SL	8.10	6.80	1.30	19.13	9.26**
7	HLW	66.03	61.69	4.34	7.03	11.77**
8	TKW	50.29	41.26	9.03	21.88	11.19**
9	GY	4021.92	2347.61	1674.30	71.32	11.56**
10	BY	13156.82	7420.37	5736.45	77.31	11.83**
11	SPW	2.54	1.78	0.76	43.06	10.72**
12	NKPS	49.00	32.13	16.86	52.49	9.47**
13	SWPS	2.18	1.54	0.65	42.21	10.65**

Table 7a. Performance of top ten percent high yielding barley genotypes under acidic soil environments at Holeta and Jeldu testing locations.

Genotype	ID	DTH	DTM	GFP	PHT	FT	SPL	HLW	TKW	GY	BMY	SPW	NKPS
16726-A	23	73.5	130.0	56.0	108.5	2.1	7.3	63.7	42.9	4722.8	14817.4	2.5	43.1
IAR/H/485	297	76.8	137.0	58.8	111.7	2.3	7.1	64.2	43.7	4663.1	14706.1	2.4	49.2
HB-1307	303	68.0	131.5	61.0	97.4	2.4	6.3	62.5	44.5	4635.7	14431.6	2.2	42.7
Shege	301	78.0	132.8	54.8	108.3	1.9	7.3	62.3	47.1	4632.9	14459.0	2.7	51.0
222969-C	192	77.6	131.7	54.1	106.1	2.2	6.5	62.5	39.5	4510.4	14336.6	2.3	49.0
HB -1966	319	68.6	131.3	60.6	93.2	2.0	6.0	64.1	45.4	4420.5	13160.6	2.2	42.5
24987-B	86	70.5	128.8	57.3	91.8	2.5	6.5	62.6	37.0	4363.9	12423.7	2.0	40.5
16737-B	25	69.3	131.6	60.1	95.2	2.4	5.4	62.8	39.8	4342.0	12858.4	2.5	49.0
17148	42	69.3	134.0	62.0	94.9	2.1	6.1	64.3	45.6	4303.0	12210.7	2.1	40.4
212947-A	137	73.4	132.0	57.5	104.3	1.9	6.8	63.5	41.0	4095.7	12304.8	2.3	47.3
1773-C	5	86.0	138.4	52.9	107.0	2.4	6.7	63.3	42.8	4083.0	13475.7	2.7	49.7
16739-D	28	70.0	129.1	57.8	91.9	2.6	7.0	63.7	41.4	4038.4	12002.4	2.1	39.4
242093-A	270	78.9	136.8	57.0	106.9	2.1	6.6	64.5	42.2	4008.8	12430.1	1.9	45.2
235541-A	240	70.6	130.4	58.2	89.5	2.1	5.2	62.7	38.3	3981.0	11177.4	1.9	43.0
EH 1493	296	73.2	130.4	56.6	92.1	2.0	6.7	63.9	43.2	3968.9	11716.9	2.2	44.1
3514-A	7	68.6	132.0	61.1	93.3	2.1	6.3	63.8	43.8	3964.6	11084.0	2.2	44.5
Ardu12-60	298	79.3	135.3	55.6	101.6	2.1	6.6	64.3	41.3	3918.1	13052.4	2.3	49.0
64111-B	94	76.5	131.7	54.9	104.7	2.2	7.3	62.3	44.1	3913.4	13199.8	2.1	36.1
24987-A	85	70.3	131.3	59.1	93.5	2.3	5.4	62.1	38.8	3902.8	10438.3	2.4	49.9
24990	88	68.8	131.0	60.1	94.0	1.9	6.2	64.9	44.3	3835.8	11698.2	2.2	37.8
24965-C	80	73.5	132.5	57.8	97.4	2.2	7.4	62.3	45.4	3777.7	13094.5	2.0	33.7
236823-A	247	74.9	131.5	56.0	104.4	2.3	7.2	62.6	42.2	3732.1	10149.2	2.2	38.6
Ibon174/03	318	58.8	123.7	61.9	83.9	2.9	6.7	65.7	48.2	3724.2	9724.2	1.5	23.1
64116-A	95	75.7	130.8	54.9	104.0	2.2	7.4	63.9	43.4	3712.1	12792.5	2.2	38.5
208836-D	124	70.2	131.0	59.1	93.6	2.0	5.5	63.0	38.8	3710.8	10749.4	2.3	48.0
24970	82	68.2	133.4	62.2	96.6	2.1	6.4	63.7	43.8	3702.1	10338.0	2.1	43.3
1773-B	4	79.3	130.0	51.6	105.5	2.1	6.5	62.0	39.8	3699.5	13637.9	1.9	41.2
Balemi	314	75.8	132.4	55.9	109.6	2.1	7.8	63.6	48.4	3690.2	13896.0	1.8	25.2
24988-A	87	69.4	129.1	58.3	94.0	2.2	6.0	63.0	39.3	3683.0	9214.5	2.4	47.4
16737-E	26	70.5	129.3	57.6	90.7	2.1	5.1	62.1	39.6	3673.2	10810.6	2.3	44.5
235551-B	243	70.8	129.8	57.9	94.1	1.9	5.6	62.3	37.5	3658.1	10674.7	2.4	51.6
16862-B	36	74.0	132.7	57.3	105.3	2.4	8.0	63.8	47.6	3644.5	12901.1	1.8	24.8
Mean		66.2	120.1	54.0	93.8	2.2	6.8	61.7	41.3	2347.8	7420.5	1.8	32.1
Minimum		51.1	98.8	46.3	64.0	1.5	4.2	57.1	30.9	1142.8	3614.2	1.1	18.0
Maximum		87.1	138.4	62.2	113.7	2.9	9.2	71.5	54.9	4722.8	14817.4	2.9	54.5

Traits[1] (refer to table 4b)

Table 7b. Performance of top ten percent high yielding barley genotypes under non-stress environments at Holeta Jeldu and Midakegn testing locations.

Genotype	ID	DTH	DTM	GFP	PHT	FT	SPL	HLW	TKW	GY	BMY	SPW	NKPS
222969-C	192	77.0	130.6	55.8	115.0	2.7	6.9	65.0	44.3	5932.8	16082.2	3.1	52.3
16726-A	23	73.7	130.2	57.3	113.8	3.0	6.9	64.7	43.8	5604.7	15026.6	2.6	44.1
Ardu12-60	298	76.1	133.2	57.4	113.0	3.2	6.8	64.3	42.5	5264.6	14695.5	3.2	54.5
24970	82	67.1	131.3	61.6	99.9	2.9	6.0	64.8	47.0	5224.1	13287.1	2.5	44.6
235551-B	243	69.7	127.6	58.2	96.7	2.6	5.1	64.5	40.1	5200.0	12737.1	3.0	54.1
HB-1307	303	68.0	127.3	59.4	103.0	3.2	6.4	64.8	47.6	5174.4	12813.7	3.1	47.2
1773-C	5	83.5	135.4	54.6	111.7	2.7	6.6	64.4	45.4	5160.5	15486.7	2.8	48.6
16739-D	28	68.5	124.4	57.1	100.7	2.9	7.0	64.4	42.5	5020.5	13482.0	2.4	43.9
EH 1493	296	71.7	129.0	57.6	99.9	2.8	7.3	65.8	44.3	4976.9	12265.0	3.0	44.2
24987-B	86	69.6	128.9	58.9	99.0	3.1	6.8	65.1	39.0	4965.8	12589.5	2.6	48.7
17148	42	68.5	131.3	61.6	97.7	2.5	6.0	65.2	46.8	4915.0	12769.3	2.7	45.3
212947-A	137	71.5	132.4	60.0	113.7	2.8	7.1	64.2	44.7	4821.2	13027.8	3.2	54.0
233040-A	220	74.7	126.6	55.4	106.0	2.6	7.8	64.5	42.2	4813.1	13011.0	3.2	50.0
24639-B	289	79.5	133.2	55.9	110.3	2.7	8.6	63.0	43.3	4812.9	12582.1	3.3	53.1
242093-A	270	75.9	132.3	57.7	114.3	2.8	7.1	65.2	43.7	4805.9	13519.3	3.2	51.7
24955-B	77	69.7	127.5	57.6	106.3	3.4	8.2	64.6	53.4	4797.6	12930.1	1.9	23.9
IAR/H/485	297	77.9	133.4	57.5	114.0	3.0	6.9	64.8	43.9	4797.1	13235.0	2.7	47.6
235541-A	240	69.8	128.1	57.9	94.2	2.8	4.9	64.3	39.7	4787.2	12335.3	2.4	48.1
236819	245	69.0	128.7	59.2	105.9	2.9	6.5	64.4	44.8	4763.1	11970.8	2.8	49.2
24967-C	81	69.7	128.1	58.7	96.0	2.9	4.9	64.4	39.0	4709.1	12736.3	2.6	51.3
Cross41/98	305	75.3	128.7	55.7	103.5	2.6	7.3	65.5	44.8	4704.9	12256.4	2.8	48.2
24990	88	71.5	131.7	60.1	99.3	2.7	6.2	65.3	47.0	4702.0	12148.5	2.5	44.0
16737-B	25	69.3	130.2	60.1	97.0	2.8	5.2	64.9	41.0	4699.4	11589.7	2.8	50.5
3514-A	7	68.0	130.2	60.7	99.8	2.8	6.5	64.3	45.8	4665.3	11075.2	2.8	48.8
236823-A	247	73.4	129.7	57.3	113.0	2.7	7.3	64.5	48.0	4636.5	14575.4	2.6	40.5
24639-A	75	79.3	132.4	55.6	111.7	2.8	8.4	62.0	43.6	4616.2	13785.5	3.2	51.2
1773-B	4	79.5	131.9	54.8	110.5	2.7	6.0	64.3	43.4	4587.9	14319.1	2.7	45.7
Shege	301	77.1	132.8	56.9	116.6	2.6	7.5	63.9	47.1	4563.9	13812.9	3.8	55.0
64144-C	99	72.1	131.1	58.9	105.5	2.8	7.3	64.8	46.4	4548.2	13637.0	2.8	42.5
4492-D	15	73.7	129.5	57.7	118.6	3.0	7.6	64.4	52.3	4500.0	13613.9	2.3	33.9
16739-B	27	70.2	126.0	57.3	106.8	3.5	7.9	64.3	53.9	4471.6	12836.0	1.7	23.0
204802-B	122	78.8	132.4	55.9	106.5	2.7	7.0	64.3	44.4	4461.1	12146.0	3.0	42.5
Mean		64.5	119.4	56.7	103.3	3.0	7.0	63.9	44.5	3212.4	8748.8	2.1	35.6
Minimum		51.03	103.57	51.91	65.34	2.38	4.18	61.14	32.54	1791.54	5104.92	1.12	19.11
Maximum		83.61	136.49	63.90	121.42	3.79	9.03	68.94	58.59	5932.80	16082.20	3.99	60.55

Traits[1] (refer to table 4b)

However, 230619-B(209), 237021(251), 221325(109), 18304-C(60), 202850-B(118), 15271(19), 242098-A(271), 234308-A(227), 239519-B(262), 234312-B(228), 230631-C(212), 235252(234), 235262(235), 232216(217) genotypes including improved varieties HB-42(299), Derebie(309), Explorer(300) were among susceptible genotypes to acid soil stress as indicated in stress score and were also located near the stress indices (SSI and TOL) and correlated negatively with yield under both soil conditions (Fig.4 and 5). Besides, these genotypes were characterized by high TOL, SSI and stress score values. High values of these indices indicate the relative sensitivity of genotypes to stress (Rosielle and Hambling, 1981). Moreover, the susceptibility of some improved barley varieties to acid soil stress was also reported earlier by Getachew *et al.* (2019).

The stress tolerance index (STI) is considered a criterion for selecting a stress tolerant genotype. High STI value indicates high tolerance and high yield potential (Fernandez, 1992), and genotypes with lower SSI values less than unity are more stress tolerate (Amsal *et al.,* 2001; Kemelew and Alemayehu, 2011; Saad *et al.,* 2014; Negarestani *et al.,* 2019).

In the first quadrant (I) of the biplot (Fig. 5), genotypes with the loading of high PC1 and low PC2 scores were characterized as high yielding with good stress tolerance as well as low-stress score values. Likewise, those with intermediate values of both components had high grain yield and tolerance, those with low PC1 and high PC2 scores had high grain yield and susceptibility whereas those with low values of both components showed intermediate grain yield and susceptibility. Similarly, in the fourth quadrant (IV) genotypes with the loading of high PC1 and PC2 score were characterized as intermediate yielding and acid soil stress tolerant, genotypes with the loading of high PC1 and low PC2 were high yielder and tolerant to stress while those with low PC1 and high PC2 were low yielding and stress susceptible. Generally, genotypes that were associated with TOL and SSI were also characterized with high values of these indices as an indicator of sensitivity to acid soil stress (Fig. 5). Khalili *et al*. (2016) also used PCA to identify tolerant genotypes to moisture stress in barley. Likewise, the selection of genotypes that have high PC1 and low PC2 are suitable for both stress and non-stress environments (Golabadi and Maibody, 2006; Teklay *et al.,* 2020). Thus, considering the biplot genotypes with larger PC1 and lower PC2, scores are characterized as high-yielding (stable genotypes), and genotypes with lower PC1 and larger PC2 scores are low-yielding or unstable genotypes (Drikvand *et al.,* 2012). Generally, genotypes with both low PC1 and PC2 have low sensitivity to stress conditions but inherently have low yield potential whereas genotypes with low PC1 and high PC2 exhibit inferior yield performance and high sensitivity to stress (Teklay *et al.,* 2020).

Conclusion

The current study confirmed the severity of acid soils in barley growing areas as depicted by the percentage of yield loss under acid soil stress as compared to non-stress experiments. Moreover, this study also revealed the existence of adequate levels of genetic variation in Ethiopian barley landraces under both acid soil stress and non-stress conditions indicating the potential for future barley genetic improvement. Therefore, the development and deployment of acid soil tolerant genotypes would be a sustainable and cost-effective strategy for resource-poor farmers. Accordingly, the currently identified high-yielding and tolerant barley genotypes need to be utilized for further adaptation studies and simultaneous breeding line extraction for subsequent crossing works and variety development. Furthermore, the national barley breeding program should effectively exploit variabilities available in Ethiopian barley landrace collections through further screening under critical acid soil environments.

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