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Ethiopian barley landraces show higher yield stability and comparable yield to improved varieties in multi-environment field trials

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Barley (*Hordeum vulgare* L.) is a major food crop in Ethiopia. A high inter-annual rainfall variability, concomitant variable planting dates and unpredictable drought stress at any time during the rainy season are severe constraints to barley production in Ethiopia. To study genotype by environment (G x E) interactions and grain yield stability, 18 barley genotypes (three landraces and 15 improved cultivars) were evaluated for yield and flowering time in two locations (Ambo and Jimma) and four staggered sowing dates over two years (2012-2013) giving a total of 16 environments. It was observed a wide phenotypic variation over environments for both grain yield (677-2,944 kg ha^{-1}) and days to 50% flowering (63-82 days). Considering the 18 genotypes and 16 environments, both genotype (G) and G x E interaction variance components were highly significant for grain yield, with a ratio of approximately 1:1. The G x E analysis revealed that the first two interaction principal component axes (IPCA1 and IPC2) in an additive main effect and multiplicative interaction (AMMI) model explained 66.1% of the total G x E interaction for grain yield (P < 0.001). Of the 16 environments, 12 grouped into two clusters which largely corresponded to test locations. The tested genotypes revealed a wide variation for both static and dynamic yield stability measures. Compared to improved cultivars, farmers' landraces displayed higher average static stability (e.g. IPCA1; P = 0.017) and similar superiority indices (dynamic stability). These landraces are therefore a source of germplasm for breeding resilient barley cultivars. Staggered planting proved to be a useful method for evaluating genotype stability across environmental factors beyond location and season.

Key words: G x E interaction, additive main effect and multiplicative interaction (AMMI), stability, landrace, barley, Ethiopia.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a major cereal crop in Ethiopia and accounts for 8% of the total cereal production based on a cultivation area of 1,018,753 ha in 2013 (CSA, 2013). Ethiopia is a center of barley diversity (Lakew et al., 1997) with a high level of morphological variation between landraces that resulted

from adaptation to diverse climatic conditions and soil types. Long- term geographic isolation likely contributed to this diversity (Mekonnon et al., 2014) because barley is a founder crop of Old World agriculture and may have been cultivated in Ethiopia for the last 5,000 years (Bekele et al., 2005). In the present time, farmers cultivate barley in Ethiopia from 1,400 to over 4,000 meters above sea level (m.a.s.l) under highly variable climatic and edaphic conditions (Asfaw, 2000). Barley is used as food, fodder and beverage in more than 20 different ways, which reflects its cultural and nutritional importance (Shewayrga and Sopade, 2011; Abraha et al., 2013). One key challenge in barley breeding is to develop varieties that are able to face the challenges of changing climatic conditions and agricultural systems.

A frequent goal of plant breeding for areas with limited resources for agricultural inputs is to produce varieties with higher average yield across diverse environments. Genotype by environment (G x E) interactions, however, frequently interfere with the selection of widely adapted genotypes (Ceccarelli and Grando, 1997). Although the breeding of varieties adapted to specific environments and cultivation practices is an alternative strategy to address the problem of low yield, changing weather patterns during periods of crop cultivation require the development of varieties with high yield stability in fluctuating environments. This notion is supported by 40 years of meteorological data, which indicate a decrease in rainfall from June to September (the main cropping season in most parts of Ethiopia) in the south western and central parts of Ethiopia (Cheung et al., 2008). As a consequence, temperature and rainfall extremes may differ substantially between locations (Mekasha et al., 2014).

Landraces represent over 90% of the cultivated barley diversity of Ethiopia (Hadado et al., 2010), and reflect a deeply rooted and ancient relationship between barley and Ethiopian farmers. So far, the national agricultural system did not deliver significantly better performing cultivars that are suitable for the cropping system of resource-poor smallholder farmers and may replace landraces (Mulatu and Lakew, 2011). Therefore, knowledge about the yield stability of existing Ethiopian barley varieties and landraces under changing environmental variables is important for the future development of barley varieties. Moreover, although barley landraces are widely cultivated in Ethiopia and considered to be an important source of genes for stability traits, information about their yield stability across variable environments is currently very limited in the scientific literature.In an eco-geographically diverse environment like Ethiopia, crop production is highly dependent on the timing of local growth seasons, and on the distribution and total amount of rainfall. Farmers may face unpredictable rainfall and drought stress patterns such as terminal drought where rainfall ends before crops have completed their physiological maturity (Cheung et al., 2008), which then poses a challenge to crop production. The absence of efficient weather forecasts and a lack of efficient communication channels for resource-poor farmers ask for the development of varieties that are robust to such irregularities. Therefore, it is useful to evaluate the robustness of barley varieties against late onset and early termination of rainfall.

In this study, our main goal was to test whether a staggered planting date in different locations and years allows identifying genotypes with low G x E and stable yields. We used this approach to compare the yield performance of a diverse set of Ethiopian barley landraces and improved cultivars and to test for differences in the environmental stability between the two groups.

MATERIALS AND METHODS

Genetic material

A total of 18 Ethiopian barley genotypes consisting of 15 improved cultivars and three landraces were included in the experiment. The cultivars and one widely used landrace were obtained from Holetta Agricultural Research Center (HARC) of Ethiopia and two local landraces were obtained from barley growers at Jimma and Ambo, respectively. The landraces represent the dominant landraces of the region. The improved cultivars were chosen based on their diversity in adaptation and genetic background. They are grown in different parts of the country and differ in traits like stress tolerance and grain yield (Table 1).

Description of the study area

The experiment was conducted at two locations in Ethiopia, Ambo and Jimma that differ in altitude, soil type and land coverage, mean annual rainfall and other characteristics (Table 2).

Definition of environments

We defined the different environments as combinations of two locations (Jimma and Ambo), two seasons (2012, 2013) and four sowing dates (done in approximately 15 day intervals between mid-June and end of July in each year), resulting in a total of 16

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Table 1. Summary of Ethiopian barley genotypes used in the study.

Code	Name	Selection history	Desirable traits of the variety other than yield
G1	Dribie	Selection from ICARDA germplasm	Tolerant to drought
G2	Agegnehu	Released cultivar derived from a landrace accession # 218950 obtained from the Ethiopian Institute of Biodiversity (EIB) through pure line selection	Tolerant to major barley leaf diseases (*Pyrenophora teres* and *Rhynchosporium secalis*) and adapted to low moisture areas
G3	Biftu	Released cultivar derived from a farmers variety 'Shasho' through pure line selection	Early vigor and tolerant to shoot fly (*Delia flavibasis* Stein) and suitable for both main and short seasons
G4	Estayish	Released cultivar derived from a landrace accession # 218963 obtained from EIB through pure line selection	High quality grain (white seeded), high market value
G5	Meserach	Released cultivar derived from a farmers' variety 'Kulumsa' through pure line selection	Early maturing and tolerant to major leaf diseases (*R. teres* and *R. secalis*)
G6	Shedeho	Released cultivar derived from a landrace accession # 3381 obtained from EIB through pure line selection	High quality grain (white seeded), high market value
G7	Misccal 21	Selection from ICARDA germplasm and released as dual purpose barley (food and malt)	High yielding with good malting quality; resistance to lodging with multiple disease resistance
G8	HB42	Released cultivar, a cross made at Holetta from IAR/H/81/ Composite 29 //Compound14/20 / Coast	Resistant to scald (*R. secalis*) and good biomass yield
G9	EH1493	Released cultivar, a cross made at Holetta from white sasa/ Composite 29//white sasa	High yielding, late maturing
G10	HB1307	Released cultivar, a cross made at Holetta from Awura gebs-1/IBON 93/91	High yielding, lodging resistant, resistant to leaf diseases *(P. teres* and *R. secalis)* with good biomass yield and white seeded
G11	Jimma Local (local check)	Farmers' variety (landrace) at Jimma, Ethiopia	Early maturing
G12	Dimtu	Released cultivar derived from a landrace accession # 3369 obtained from EIB through pure line selection	Good yield under low input conditions with good biomass yield
G13	Basso	Released cultivar derived from a landrace accession # 4731 obtained from EIB through pure line selection	Suitable for main and short seasons
G14	Cross 41/98	Released cultivar, cross made at Holetta from 50-16/3316-03// HB42/Alexis	High yielding, late maturing
G15	Abay	Released cultivar derived from a landrace accession # 3357 obtained from EIB through pure line selection	High quality grain (white seeded) with long spike and medium to early maturity
G16	Ambo Local (local check)	Farmers' variety (landrace) at Ambo, Ethiopia	Suitable for main season with big grain size
G17	Balame	Dominant farmers' variety (landrace) at West Shoa, Ethiopia	Tolerant to low soil fertility and drought, good flour quality
G18	Shege	Released cultivar derived from a landrace accession # 3336 obtained from EIB through pure line selection	Good yield under low input conditions and tolerant to major leaf diseases (*P. teres* and *R. secalis*)

environments (Table S1). No serious moisture stress was experienced right after all four sowing dates in the two seasons and locations, except at the fourth sowing date at Jimma in 2012. In both years, the rainy season finished earlier at Ambo than Jimma (Figure S1).

Experimental design

A randomized complete block design (RCBD) was used for each combination of location, season and sowing date. The dimension of a single plot was 2.4 m width and 2.5 m length (6 m^2) and it

Table 2. Characteristics of the two test locations in Ethiopia.

Characteristics	Location	
	Ambo	Jimma
Position relative to Addis Ababa	135 km West	365 km Southwest
Latitude	8°57'N	7°42'N
Longitude	37°45'E	36°48'E
Altitude (m.a.s.l.)	2,005	1,790
Mean annual rainfall (mm, average over 20 years)	1,041	1,625
Min., Mean and Max Temperature (°C) over 20 years	10.2, 18.0 and 26.3	11.3, 18.5 and 26.5
Soil type	Clay	Clay loam
Soil organic matter (%)	5.14 - 5.54	5.93 - 6.33
Soil cation exchange capacity (meq/100 g soil)	36.0 - 37.2	31.6 - 33.8
Soil pH (Gerba et al., 2013)	6.63 - 6.85	6.11 - 6.19
Land coverage	Crops like wheat, barley and maize	Denser in forest coverage as part of tropical rainforest
Total rainfall (mm) in the 2012 growing season (June-December)	894	880
Total rainfall (mm) in the 2013 growing season (June-December)	887	1,036

was planted with 12 rows at a distance of 0.2 m between, which corresponded to Holetta Agricultural Research Center **(**HARC) recommendations.

Trial management

Fifty-one grams of barley seeds were manually drilled per plot as recommended by HARC. Fertilizer was applied to each trial field as 100 kg diammonium phosphate (DAP) and 50 kg urea per hectare split into two time points. 15 g of Urea and 30 g of DAP were added to a plot at time of sowing and the same amount at the tillering stage. The trial plots were weeded by hand.

Data collection

The traits measured were grain yield (kg ha^{-1}) and days to 50% flowering. To measure grain yield, matured spikes were harvested from ten inner rows of each plot when the seeds were matured. The spikes were then further dried and threshed. The clean seeds were dried in the oven until the moisture content was zero to avoid a bias in moisture content between different harvests. The yield was adjusted to 12.5% moisture content in kg ha^{-1}. To determine days to 50% flowering, the date was counted from sowing to 50% of the spikes were completely emerged from the leaf sheaths in a plot based on visual assessment.

Statistical analysis

The grain yield data were analysed with GenStat for Windows 17th Edition (VSN International, 2014). A two-way ANOVA determined the effect of environment on grain yield, and a four-way interaction ANOVA was carried out to examine the main and interaction effects of factors on grain yield with the following model:

$$X_{ijklm} = \mu + Y_i + G_j + L_k + S_l + (YG)_{ij} + (YL)_{ik} + (GL)_{jk} + (YS)_{il} + (GS)_{jl} + (LS)_{kl} + (YGL)_{ijk} + (YGS)_{ijl} + (YLS)_{ikl} + (GLS)_{jkl} + (YGLS)_{ijkl} + \varepsilon_{ijklm} \quad (1)$$

Where X_{ijklm} = the value of treatment in the i^{th} Year, j^{th} Genotype, k^{th} Location, l^{th} Sowing date and m^{th} replication; μ = grand mean; Y_i = ith Year; G_j = j^{th} Genotype; L_k = k^{th} Location; S_l = l^{th} Sowing date; $(YG)_{ij...}$ = interactions between Year, Genotype, Location and Sowing date etc.; and ε_{ijklm} = error of X_{ijklm}.

An additive main effect and multiplicative interaction (AMMI) model was used to dissect the G x E interaction (Gauch, 1992) using the Meta Analysis function in GenStat. Each combination of location, season and sowing date was considered as an environment giving a total of 16 environments. The AMMI model for 18 genotypes and three replications was defined as (Gauch 2013):

$$Y_{ijr} = \mu + \alpha_i + \beta_j + \sum \lambda_k \gamma_{ik} \delta_{jk} + \rho_{ij} + \tau_{r(e)} + \varepsilon_{ijr} \quad (2)$$

where Y_{ijr} = yield of the i^{th} genotype in the j^{th} environment for replicate r, μ = the grand mean, α_i = the genotype deviation from the grand mean, β_j = the environment deviation, λ_k = the singular value for the interaction principal component (IPC) k, γ_{ik} = the eigenvector value for genotype i and component k, δ_{jk} = the eigenvector value for environment j and component k, ρ_{ij} = the residual, $\tau_{r(e)}$ = the block effect for replication r within environment j, and ε_{ijr} = the error.

Stability analysis

The static and dynamic yield stability concepts describe the differential response of genotypes to variable environments (Becker and Leon, 1988). Under the static stability concept, the yield performance of genotypes remains constant in different environments, whereas under the dynamic stability concept the response of a stable genotype to the environment is parallel to the average response of all genotypes in the trial (Becker and

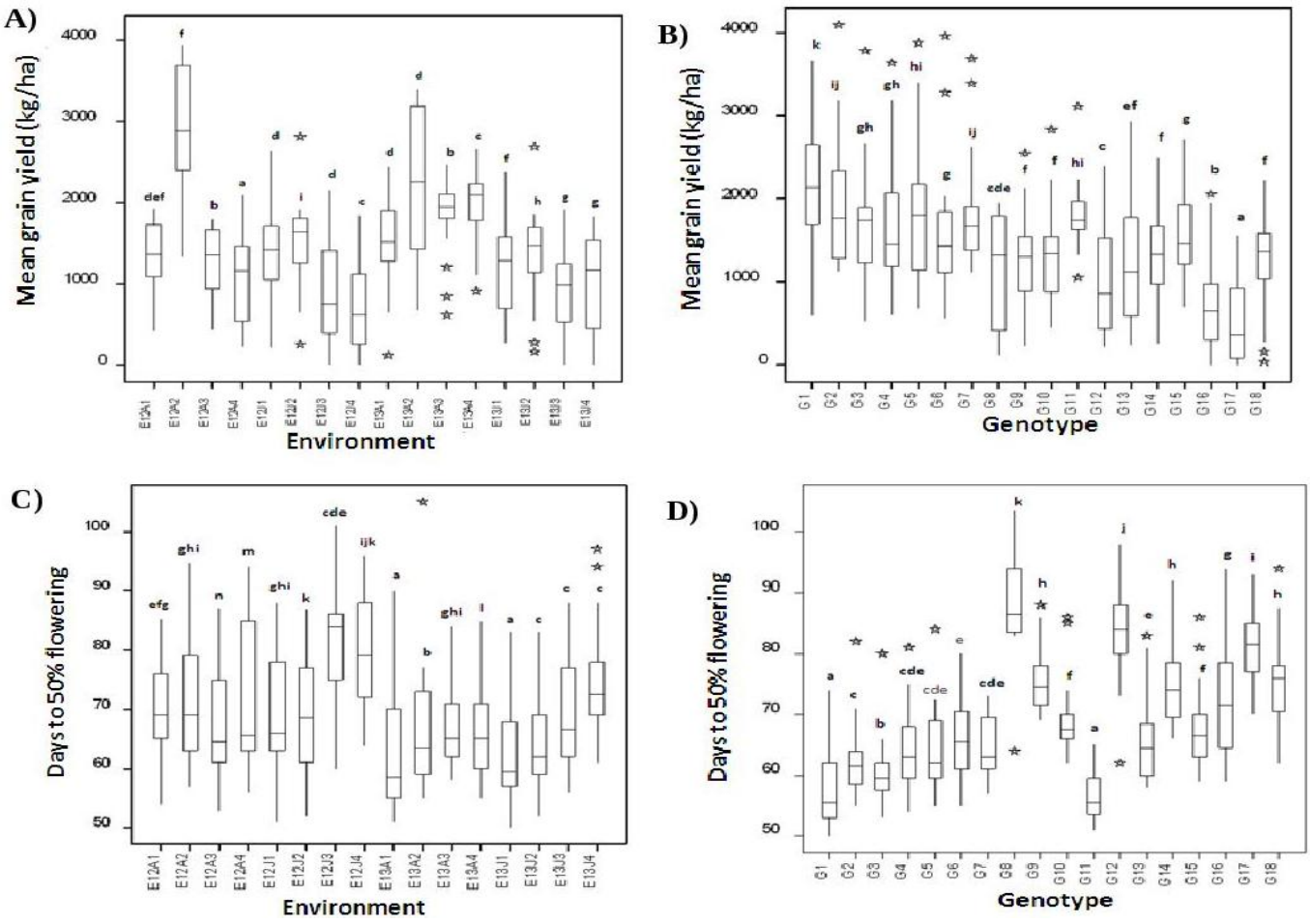

Figure 1. Boxplots for mean grain yield (A and B) and days to 50% flowering (C and D) as affected by the 16 environments and 18 genotypes, respectively. Individual letters (a-n) above each box plot shows significant differences and box plots with different letters are significantly different ($P < 0.05$) from each other. Stars above box plots indicate outliers.

Leon, 1988). We estimated the following stability indices with GenStat:

(1) Superiority index (SUP): This index, proposed by Lin and Binns (1988), measures the distance in grain yield of a given genotype to the genotype with the maximum performance in each environment. It consists of a non-parametric analysis, which is simpler and addresses the limitations of a linear regression analysis (Oliveira et al., 2013). A small SUP value indicates a better fit of a genotype to the dynamic stability concept.
(2) Static stability coefficient (SSC): This index measures the consistency of genotype performance for grain yield. It is based on environmental variances i.e. the variance of yields of each genotype over test environments (Lin et al., 1986; Becker and Leon, 1988). A low value (closer to zero) of this coefficient indicates a better fit of a genotype to the static stability concept.
(3) The first interaction principal component axis (IPCA1): IPCA1 values obtained from the AMMI model indicate the position of genotypes on an AMMI biplot. Genotypes with an absolute value close to zero have a higher static stability.
(4) AMMI stability value (ASV): This value is calculated from the IPCA1 and IPCA2 scores of each genotype in the AMMI model and the two main principal component axes (PC1 and PC2; Zali et al., 2012). This parameter also follows the static stability concept and ranks genotypes with low values as more stable (Purchase et al., 2000).

To test for differences in the stability parameters between landraces and cultivars, we used the Mann-Whitney U (Wilcoxon rank-sum) test.

RESULTS

Environmental means, repeatability and differentiation among entries

The field trials revealed a strong effect of the environment on grain yield (Figure 1A). Environmental means for grain yield differed between the 16 environments and ranged from 677 to 2,944 kg ha^{-1},

Table 3. ANOVA showing the effects of genotypes, environments and G x E interaction on grain yield and days to 50% flowering of 18 barley varieties grown in 16 environments (location-season-sowing date combinations in Ethiopia).

Source of variation	D.F	Grain yield		Days to 50% flowering	
		MS	Variance	MS	Variance
Genotype	17	8,479,572***	165,140	3,584.1***	73.0
Environment	15	17,750,843***	318,481	1.515.5***	26.6
G x E	255	552,873***	151,831	81.7***	22.0
Error	574	97,383	97,383	15.8	15.8
Total	863				

DF, degree of freedom; MS, means squares. ***significant at P < 0.001 probability level.

with an overall mean of 1,447 kg ha^{-1} (2-way ANOVA, P < 0.001; Table 3 and Table S2). Pair wise comparisons of factors revealed that (i) the later (fourth) sowing date produced lower yields (1,191 kg ha^{-1}) than the earlier (first) sowing date (1,364 kg ha^{-1}; t-test, P < 0.05); (ii) genotypes performed better at the Ambo site (1,873 kg ha^{-1}) than at the Jimma site (1,182 kg ha^{-1}; t-test, P < 0.001) and (iii) genotypes performed better in 2013 (1,593 kg ha^{-1}) than 2012 (1,463 kg ha^{-1}; t-test, P < 0.05). The environment also affected the number of days to 50% flowering with means ranging from 63 to 82 days (2-way ANOVA, P < 0.001; Tables 3 and S3). Late sowing caused a longer time span to 50% flowering (73 days) than early sowing (66 days; t-test, P < 0.01). Genotypes were differed for grain yield and days to 50% flowering ranging from 525 to 2,119 kg ha^{-1} for grain yield and 58 to 88 days to 50% flowering (2-way ANOVA, P < 0.001; Tables 3, S2 and S3). The three top yielding genotypes were improved varieties, whereas the lowest yielding genotype, Balame (G17) was the landrace most widely used by Ethiopian farmers. Grain yield was negatively correlated with days to 50% flowering across the 16 environments, but with variable significance levels. The correlation coefficients between grain yield and days to 50% flowering ranged from -0.33 to -0.88 (Table S3). Estimated repeatability ranged from 0.46 to 0.92 for grain yield, and from 0.52 to 0.98 for days to 50% flowering among environments (Table S2 and S3). Repeatability did not differ between locations or sowing dates.

Variance components

Genotype (G), environment (E) and genotype-by-environment (G x E) interaction affected both grain yield and days to 50% flowering (2-way ANOVA, P < 0.001). For grain yield, the ratio of G to G x E variance was nearly one (Table 3). A combined ANOVA of genetic and environmental factors revealed significant effects of G (P < 0.001), genotype-by-location (G x L; P < 0.001), genotype-by-sowing date (G x SD; P < 0.001) and genotype-by-year (G x Y; P < 0.01) for grain yield. Ratios of G variance to G x SD, G x L and G x Y interactions were about two, three and nine times, respectively (Table 4).

Level of genotype x environment interactions

The AMMI analysis of variance for grain yield and days to 50% flowering of the 18 barley genotypes evaluated in 16 environments showed that G x E had a significant effect on trait values (P < 0.001). The environment explained 48.3% of the total sum of squares implying that the environments were sufficiently diverse to differentiate between genotypes. The remaining 26.1 and 25.6% of the variation resulted from genotype and G x E effects, respectively. The partitioning of the G x E interaction revealed that IPCA1 captured 44.4% and IPCA2 21.7% of variation in grain yield. Similarly, 43.9 and 20.2% of the interaction was explained by IPCA1 and IPCA2, respectively, for days to 50% flowering. The mean squares of the two components (IPCA1 and IPCA2) were differed significantly (AMMI ANOVA, P < 0.001) and explained a total of 66.1 and 64.1% of the variance of the G x E interaction in grain yield and days to 50% flowering, respectively (Table 5 and Figure 2A and B).

Environments and genotypes showed much variation for both traits in terms of main effects and their interaction. For example, genotype G11 located close to the origin in the biplot and showed low IPCA1 and IPCA2 values suggesting little interaction with the environment and a good performance for grain yield compared to other genotypes. In contrast, G5, G8 and G18 were the most unstable genotypes because they were more distant to the origin of the biplot. With respect to the contribution of environments to G x E interactions, environments 13A3, 13A2 and 12A2

Table 4. Combined ANOVA showing mean square and variance components of grain yield and days to 50% flowering of 18 barley genotypes in 2012 and 2013.

Source of variation	D.F	Grain yield		Days to 50% flowering	
		MS	Variance	MS	Variance
Genotype (G)	17	8,479,572***	152,320	3,584.0***	74.0
Location (L)	1	92,588,723***	209,549	3,586.0***	6.1
Year (Y)	1	3,291,308**	6,248	7,995.0***	15.5
Sowing date (SD)	3	26,326,767***	144,169	2,480.0***	8.9
G x Y	17	592,165**	16,611	100.2***	0.1
G x L	17	2,063,589***	46,886	141.1***	1.8
G x SD	51	505,003***	87,994	60.8***	8.4
G x Y x L	17	304,863***	5,285	96.8***	1.3
G x Y x SD	51	463,031***	93,197	85.9***	1.2
G x L x SD	51	487,641***	84,449	81.6***	12.6
G x Y x L x SD	51	321,820**	132,557	67.5***	25.9
Residual	574	97,383	97,383	15.8	15.8
Replication	2	153,190		26.4	
Total	863				

DF, degree of freedom; MS, means squares. *, **, ***Significant at P < 0.05, P < 0.01, P < 0.001 probability level, respectively.

Table 5. ANOVA of the AMMI model with 18 barley genotypes based on grain yield and days to 50% flowering in 16 environments.

Source of variation	Grain yield			Days to 50% flowering		
	DF	MS	% explained by IPCAs	DF	MS	% Explained by IPCAs
Treatments	287	1,921,248***		287	364.1***	
Genotype (G)	17	8,479,572***		17	3584.1***	
Environment (E)	15	17,750,843***		15	1515.5***	
Block	32	140,171*		32	12.7	
G x E	255	552,873***		255	81.7***	
IPCA 1	31	2,018,458***	44.4	31	295.1***	43.9
IPCA 2	29	1,056,456***	21.7	29	145.3***	20.2
IPCA 3	27	407,506***	7.8	27	137.7***	17.8
IPCA 4	25	409,232***	7.3	25	50.9***	6.1
IPCA 5	23	300,351***	4.9	23	44.5***	4.9
IPCA 6	21	304,051***	4.5	21	25.4*	2.6
IPCA 7	19	231,757***	3.1	-	-	-
Residual	80	110,541		99	7.2	
Error	544	95,072		544	16.0	
Total	863	704,058		863	131.7	

DF, degree of freedom; MS, mean Squares; IPCA, interaction principal component axis. *, **, ***Significant at P < 0.05, P < 0.01, P < 0.001 probability level, respectively.

contributed most as indicated by their distance to the origin in the biplot (Figure 2A) and allowed a better discrimination of genotypes. Environments 12J3, 12J4, 12A3 and 13A4 had the least effect on G x E interaction.

Among the 16 environments, 12 grouped into two clusters of seven and five environments, with a clear separation to the remaining four environments (Figure 2A). All environments except one of the first cluster were located in Jimma and showed high repeatability values ranging from 0.64 to 0.91. The environments of the second cluster were all located in Ambo (with one

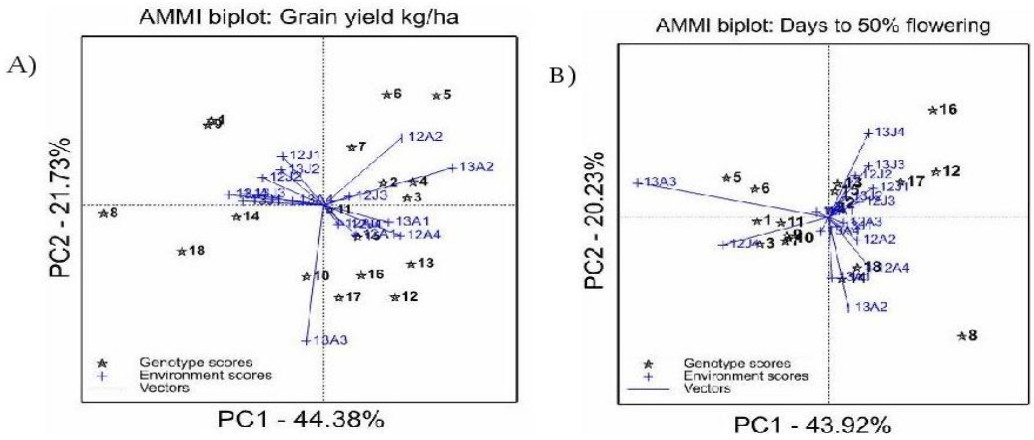

Figure 2. AMMI biplots showing relationships among 18 barley genotypes and 16 environments (location-season-sowing date combinations in Ethiopia) for grain yield (A) and days to 50% flowering (B).

exception) and also showed a high repeatability ranging from 0.63 to 0.92.

Estimation of stability parameters and difference between cultivars and farmers' landraces

Superiority index (SUP) values ranged from 149 to 1,969, and static stability coefficient (SSC) values from 211 to 791. They indicate large differences among tested genotypes for both dynamic and static yield stability (Table 6). Based on three static stability parameters, the three landraces had a higher static stability because the overall average rank was 4 for the landraces and 11 for the modern cultivars. Significant differences between the landraces and the modern cultivars were observed for the three static stability parameters SSC, IPCA1 and ASV, but not for the dynamic stability parameter SUP (Table 7).

DISCUSSION

Relative effects of location, year and sowing dates on grain yield

The two locations for the field trials were selected on the basis of their differences in agro-ecological features. Ambo represents a temperate, intermediate highland region with intensified barley production. The area is mainly known for the production of cereals like barley and wheat (Mengistu, 2010). Jimma is located in the hot and humid zone of tropical rain forest of the southwestern part of Ethiopia. Since its elevation is in the mid-altitude range, it is characterized by denser tree coverage. The main crops of this region are maize and sorghum, although wheat and barley are also produced (Yisehak, 2008). As shown in Figure S1, the two locations differ in the pattern of rainfall distribution, and in the minimum and maximum temperature that likely contribute to the effect of the two locations on the grain yield performance of barley.

The staggered sowing dates were chosen to include the regular date of sowing according to the local sowing calendar, but included earlier and later dates to produce a larger environmental variation, in particular drought stress at different stages of plant development, in order to evaluate diverse local conditions on yield and G x E interactions.

It was examined the overall grain yield performance of genotypes in relation to the growth conditions of the different environments. The low grain yield at Jimma ranged between 896 to 1,284 kg ha^{-1} and may result from the moisture stress experienced during the flowering stage of environments 12J3, 12J4 and 13J4 in combination with the extended rainfall during the maturity stage. Drought stress during flowering can strongly affect yield in barley (Vaezi et al., 2010). In contrast, the late sowing dates of 2013 at Ambo (13A3 and 13A4) did not result in drought stress during flowering and did not affect grain yield much, possibly because the higher amount of rain prior to the end of the rainy season was stored in the soil. Residual soil moisture contributes to the completion of developmental stages in barley and other crops (Asfaw, 2000). In general, location and sowing dates displayed highly significant effects on grain yield of barley in our set of genotypes (Table 4) and the staggered planting was seen as additional means to allow genotypes respond differently to the array of environments apart from

Table 6. Mean grain yield (kg ha^{-1}) and estimated yield stability parameters of 18 barley genotypes evaluated across 16 environments (location-season-sowing date combinations in Ethiopia).

Genotype	Grain yield		SUP		SSC		IPCA1		ASV	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank
G1	2119	1	149*	1	522*	10	-19.3	14	39.6	14
G2	1922	2	204	2	657	15	10.3	7	21.2	7
G3	1749	6	395	7	566	12	14.5	10	29.6	8
G4	1695	7	368	6	664	16	15.5	13	31.0	10
G5	1819	5	333	5	791	18	19.5	15	40.0	16
G6	1516	8	398	8	780	17	11.0	8	22.9	12
G7	1848	3	224	3	559	11	4.9	4	10.6	3
G8	1186	15	1133	16	473	9	-37.9	18	77.3	18
G9	1317	13	840	12	395	6	-19.9	16	40.8	15
G10	1347	9	728	10	385	5	-2.9	3	7.0	4
$G11^{(LR)}$	1847	4	270	4	211	1	1.1	1	2.4	1
G12	1034	16	1115	15	580	13	12.3	9	25.6	11
G13	1262	14	905	14	618	14	15.1	12	31.1	13
G14	1330	12	742	11	321	3	-15.0	11	30.6	9
G15	1461	10	489	9	361	4	5.9	5	12.3	2
$G16^{(LR)}$	734	17	1507	17	458	8	6.5	6	13.8	6
$G17^{(LR)}$	528	18	1969	18	274	2	2.6	2	6.9	5
G18	1337	11	882	13	437	7	-24.3	17	49.8	17

LR = landraces, SUP = superiority index, SSC= static stability coefficient, IPCA1 = the first interaction principal component axis; ASV = AMMI stability value, * = numbers are divided by 1000.

Table 7. Summary of Mann-Whitney U (Wilcoxon rank-sum) test showing significant difference in static yield stability between landraces and improved cultivars.

Stability parameter	Mean rank of landraces	Mean rank of cultivars	P-value
SSC	4	11	0.039^{*}
IPCA1	3	11	0.017^{*}
ASV	4	11	0.027^{*}
SUP	13	9	0.25^{NS}

SSC, static stability coefficient; IPCA1, the first interaction principal component axis; ASV, AMMI stability value; SUP, superiority index. * significant at $P < 0.05$, NS non-significant.

location and year difference. The combination of year, location and staggered planting date efficiently creates a diversity of environments to test the environmental stability of barley genotypes. However, the effect of location was the strongest because it divided the genotypes in to two groups based on grain yield performance (Figure 2A).

Patterns of G x E interaction

The multi-environment testing of genotypes to assess G x E interactions and genotype yield stability plays an important role in either selecting widely adapted genotypes to be used across different environments, or in selecting genotypes specifically adapted to a particular sub-set of environments. In this regard, different trials assessed the differential response of barley across environments and mainly accounted for location and seasonal variation (Abdipur and Vaezi, 2014; Sarkar et al., 2014; Mehari et al., 2014). To fully exploit the differential responses of genotypes under a wider range of environments apart from location and year differences, testing genotypes at different sowing dates enables to include more environmental variables like moisture levels or atmospheric and soil temperature

regimes which also appear in farmers' fields. As expected, our trial revealed a substantial genotype-by-sowing date (G x SD) interaction ($P < 0.001$; Table 4) suggesting that genotypes differed in their ability to cope with early versus late planting dates. Understanding such patterns may allow specific variety recommendations and optimized selection of varieties by farmers, depending on the actual sowing date and given that an appropriate seed system is in place.

The dissection of G x E interactions in the current trial suggested that 12 out of the 16 environments grouped into two clusters or mega-environments. These clusters largely corresponded to the two locations, Jimma and Ambo, suggesting that genotypes that produce high grain yields in both highly distinct environments (locations) can be considered as adapted genotypes for these locations.

Specific advantages of landraces over improved cultivars

Among the genotypes investigated, 11 were pure line selections from local landraces, four resulted from crosses followed by successive selfing, and three were farmer landraces. The four stability parameters analyzed in the study were based on either the static stability concept, that is, genotypes with stable and high yield (SSC, IPCA1 and ASV) or the dynamic stability concept, that is, genotypes that respond with a higher yield if the environment improves (SUP). The G11 landrace (Jimma Local) was the most stable of all genotypes by all three static stability parameters. Another landrace (G17, Balame) was classified as the second most stable genotype by two of the three measures although the mean grain yield was not high. The landraces showed a higher static stability than improved cultivars (Table 7), which was also observed in previous studies on maize (Salazar et al., 2007), wheat (Jaradat, 2013) and field crops in general (Oliveira et al., 2013). The higher genetic diversity of landraces highly contributes to their increased stability (Ceccarelli, 1994). Since barley is mainly a self-pollinated crop, barley landraces are mixtures of mostly homozygous genotypes (Brown, 1978; Rodriguez et al., 2012) and landraces with a better mean grain yield can readily be utilized or be used as a basis for further improvement provided that static stability is considered important by the farmers and breeders. Improved cultivars like G1, G2 and G7 performed better than farmers' landraces in terms of dynamic stability (SUP), but the differences were not statistically significant. Improved cultivars usually tend to respond better to optimal environmental conditions than landraces (Pswarayi et al., 2008), and hybrids of winter barley showed a higher dynamic yield stability than lines (Mühleisen et al., 2014). The wide range of SUP values in our trial for both landraces and improved cultivars suggest that both types of varieties can be improved significantly for dynamic stability. The current study included three landraces: The dominant farmers' variety in West Shoa region of Ethiopia (Balame) and two other landraces from the location where the field experiment was conducted (Ambo Local and Jimma Local). An inclusion of more landraces from other barley growing regions might be helpful to fully investigate the relative performance in terms of grain yield and stability of improved cultivars and barley landraces in Ethiopia. However, the present results suggest that the G11 landrace (Jimma Local) is the best candidate for risk-averse farmers who prefer static stability combined with high mean yield. In contrast, genotypes G1 (Dribie), G2 (Agegnehu) and G7 (Misccal 21) are improved cultivars with a high dynamic stability and are suitable varieties for farmers favouring dynamic response to better growing conditions and providing higher inputs.

Scope for exploiting specific adaptation to factors that are known before planting

The AMMI biplot grouped the testing environments into two groups characterized by the two locations (Figure 2A), which indicates that selection needs to be done separately for the two regions if the breeding objective is specific adapting cultivars for the locations. Although the grouping was based on location, the highly significant G x L and G x Y x L, G x Y x SD interaction effects (Table 4) suggested that the selection of new barley varieties requires field trials in different and multiple years, but also at different sowing dates to asses yield stability by accounting for variation in the beginning and end of the rainfall season. This notion is supported by a study in sweet sorghum, which reported a high G x E interaction with sowing date as the largest contributor to the interaction (Reddy et al., 2014). Some genotypes performed very well in specific environments, and their specific adaptation can be attributed to *a priori* known factors like location.

The differential performance of genotypes over test environments raises the question, which traits are mainly responsible for the differences. For example, genotype G1 was identified as best overall genotype for the Jimma location because of its high yield, whereas genotype G11 exhibited the best static stability. Both were the two earliest flowering genotypes among the 18 tested (Figure 1D and Table S3). They reached the stage of 50% flowering plants on overall average at 58 (G1) and 57 days (G11) after sowing, respectively, which was 12 and 13 days earlier than the average over

all genotypes (70 days). This result and the negative correlation of grain yield and flowering time in 13 of the 16 environments indicates the importance of early flowering for yield performance and stability. Similarly, early flowering genotypes of wheat showed less yield reduction after stress than late flowering genotypes (Talukder et al., 2014), and early maturing Ethiopian barley landraces performed better than late maturing ones in a year of high season-end drought (Sinebo et al., 2010). Therefore, breeders can consider days to 50% flowering as a target trait in breeding programs aimed at yield stability.

For a breeder to choose which stability concept to apply, the inclination of farmers to take a risk is relevant. In case of a high preference of farmers to avoid risk by preferring lower but stable yield over a high yield under optimal environmental conditions and inputs, static stability parameters should be applied to selection. A dynamic stability concept can be considered as selection criterion, if farmers are willing to accept a higher risk. The barley varieties and landraces used in our study showed a wide range of both static and dynamic stability measures, which indicates the presence of genetic variation to improve both types of stability. Yield stability can be achieved by two different mechanisms, namely individual buffering and population buffering (Ceccarelli et al., 1991). Individual buffering is influenced by traits like responsive tillering, photoperiod-sensitive flowering and resistance to biotic and abiotic stress factors as was shown in pearl millet (Haussmann et al., 2012). At the population level, intra-population variation in flowering time may buffer unpredictable and unfavorable growth conditions. Such a buffering was observed in oat, where grain yield differed significantly between a mix of genotypes and the individual pure lines in response to stress (Helland and Holland, 2001). Individual buffering is frequently believed to be a property of heterozygous crops and difficult to exploit in self-pollinated diploid crops like barley (Ceccarelli et al., 1991). Since modern line cultivars are highly uniform, population buffering is not possible. Therefore, a possible strategy for barley breeding in a diverse and changing environment as in Ethiopia, is to combine different selected genotypes in a mixture, providing different trait combinations to achieve sustainable population buffering. Traditional landraces are mixture of genotypes which might explain the higher static stability observed for them in the present study.

Need to further develop the Ethiopian seed system

The current barley seed system of Ethiopia is mainly informal because of the highly diverse structure of agriculture (Abay et al., 2011). Farmers usually get seed for next season from their previous harvest, neighbors or local open markets. Commercial plant breeding or seed companies actively involved in the seed system are almost non-existent. Seeds are seldom provided by public research institutes or local agricultural extension services to barley growers though efforts have been made to create formal seed system. As location and sowing date factors are predictable ahead of planting, seeds of the appropriate cultivars must be made available to the growers on a very short-term basis, to enable exploitation of specific adaptations. This requires decentralized seed production of required cultivars, and a strengthening of the local, informal and semi-formal seed sector in Ethiopia, in order to make the seed available on time. As long as the seed sector is unable to provide on time the seed of specifically adapted cultivars, promotion of widely adapted cultivars identified by the approach used in this study is possibly the better short-term strategy to follow.

Conclusions

The analysis of 18 barley genotypes grown in 16 environments (location-season-sowing date combinations in Ethiopia) with the AMMI statistical model revealed that a staggered sowing date enabled to exploit G x E patterns beyond location and season. The major proportion of the total variation in grain yield was explained by location followed by sowing date. The year of cultivation had a smaller effect than location and sowing date as shown by the variance components. Adaptation to a specific location was detected for the G15 (Abay) cultivar, while others showed a wider adaptation. The observed G x E patterns can be exploited by barley breeders and farmers by a tactical choice of varieties to be cultivated depending on the actual location and sowing date. Landraces showed, on average, higher static yield stability than improved cultivars with a comparative grain yield. Our study showed that by including staggered planting dates in combination with different years and locations, a diversity of environments can be created to test the environmental stability of barley genotypes if resources for field trials are limited as in developing countries like Ethiopia. For further breeding efforts, the number of environmentally diverse environments has the strongest effect on the analysis of G x E interaction and the number and type of location used to select for improved varieties likely have the strongest effect in producing future-proof barley cultivars for Ethiopian agriculture.

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Conflict of Interest

The authors have not declared any conflict of interest.

Abbreviations

G, genotype; E, environment; SD, sowing date; L, location; Y, year; G x E, genotype-by-environment; G x Y, genotype-by-year; G x L, genotype-by-location; G x SD, genotype-by-sowing date; G x Y x L, genotype-by-year-by-location; G x Y x SD, genotype-by-year-by-sowing date; G x L x SD, genotype-by-location-by-sowing date; G x Y x L x SD, genotype-by-year-by-location-by-sowing date; GY, grain yield; DtF, days to 50% flowering; AMMI, additive main effects and multiplicative interaction; IPCA, interaction principal component axis; SUP, superiority index; SSC, static stability coefficient; ASV, AMMI stability value; CSA, Central Statistical Agency of Ethiopia; HARC, Holetta Agricultural Research Center.

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Supplementary Tables and Figure

Table S1. Sixteen environments used for evaluation of barley genotypes.

Code	Location	Sowing date	Code	Location	Sowing date
12A1	Ambo	June 9, 2012	13A1	Ambo	June 11, 2013
12A2	Ambo	June 26, 2012	13A2	Ambo	June 26, 2013
12A3	Ambo	July 13, 2012	13A3	Ambo	July 12, 2013
12A4	Ambo	July 28, 2012	13A4	Ambo	July 27, 2013
12J1	Jimma	June 13, 2012	13J1	Jimma	June 13, 2013
12J2	Jimma	June 28, 2012	13J2	Jimma	June 28, 2013
12J3	Jimma	July 14, 2012	13J3	Jimma	July 14, 2013
12J4	Jimma	July 30, 2012	13J4	Jimma	July 30, 2013

Table S2. Mean grain yield (kg ha^{-1}) of the18 genotypes across 16 environments.

Genotypes	Environments																Mean
	12A1	**12A2**	**12A3**	**12A4**	**12J1**	**12J2**	**12J3**	**12J4**	**13A1**	**13A2**	**13A3**	**13A4**	**13J1**	**13J2**	**13J3**	**13J4**	
1[†]	1731	3665	1665	591	2639	2812	2160	1227	1725	2318	2107	2662	2389	2687	1925	1595	2119
2	1777	4099	1254	1299	1888	1809	1407	1128	2381	3183	2466	2288	1214	1745	1539	1266	1922
3	1930	3781	1723	1086	1193	1260	1830	1847	1763	2669	1810	2145	1368	1488	532	950	1749
4	1465	3637	1432	1394	1715	1379	1303	689	2148	3185	2073	2067	1056	1705	600	1065	1695
5	1801	3879	1804	2093	2183	1892	1209	681	2440	3400	846	2160	1243	1456	943	1070	1819
6	1224	3962	1394	1463	1760	1839	799	564	1531	3279	1207	2041	1248	1850	1023	936	1516
7	1360	3689	1485	1738	1703	1916	1445	1122	1279	3390	1829	2626	1888	1652	1248	1408	1848
8	426	1881	698	395	1610	1738	323	230	121	681	1863	1951	1581	1192	1444	1839	1186
9	1218	2544	1164	232	1446	1732	392	353	1298	1421	618	2130	1480	1309	1264	1615	1317
10	1386	2837	1296	1504	1053	1513	570	704	1515	1234	2239	1828	1585	1139	696	453	1347
11	1696	3113	1747	1836	1662	1737	1431	1596	2105	2182	1832	2232	1329	1832	1049	1696	1847
12	1082	2396	715	998	685	1080	593	265	1668	2244	2387	1394	616	280	222	255	1034
13	1756	2935	1699	1233	915	1012	734	293	1302	2280	2027	1781	633	541	367	244	1262
14	1016	2501	939	537	1267	1707	590	258	1361	1685	1984	1667	1371	1295	1116	1483	1330
15	1725	2690	1474	1434	1055	1362	765	1184	1902	2724	1946	2295	697	1485	1235	1440	1461
16	736	2056	841	613	352	649	248	-	651	1916	1956	1114	463	774	-	-	734
17	553	1340	440	452	220	258	-	-	941	1284	1562	912	280	165	-	-	528
18	1252	1980	1336	275	1394	1563	157	37	1125	1331	2225	2221	1613	1503	934	1540	1337
Mean	1341	2944	1284	1065	1374	1514	887	677	1514	2245	1832	1973	1225	1339	896	1048	1447
S.E±	130	134	104	234	105	108	116	93	255	269	262	186	168	182	186	159	
LSD (5%)	374	385	300	671	302	310	332	267	734	773	752	534	483	524	535	458	
Repeatability	0.91	0.89	0.89	0.92	0.78	0.92	0.82	0.63	0.75	0.77	0.71	0.82	0.81	0.75	0.46	0.64	0.78

[†] See Table 1 for genotype codes.

Table S3. Days to 50% flowering of the18 genotypes across 16 environments.

Genotypes	Environments																Mean
	12A1	12A2	12A3	12A4	12J1	12J2	12J3	12J4	13A1	13A2	13A3	13A4	13J1	13J2	13J3	13J4	
1[†]	54	57	53	56	51	55	67	74	53	55	65	60	50	52	57	64	58
2	65	63	61	63	63	61	82	71	55	59	58	59	57	57	62	71	63
3	62	62	59	60	56	58	66	80	57	58	65	58	53	54	60	62	61
4	65	69	62	64	64	67	81	75	54	59	60	61	59	59	60	69	64
5	68	62	63	60	62	62	75	72	55	59	84	60	57	59	62	70	64
6	71	67	63	65	66	67	80	78	55	59	78	62	60	59	63	70	66
7	65	68	62	63	63	60	70	73	57	71	71	62	59	60	63	69	65
8	86	95	87	94	86	84	101	94	90	105	64	85	83	83	83	88	88
9	74	79	75	75	72	77	86	88	70	73	79	71	69	69	72	77	75
10	69	74	69	67	66	68	86	85	66	65	70	67	62	64	66	70	70
11	54	57	53	59	58	52	60	64	51	55	65	55	52	55	56	61	57
12	81	86	81	87	88	85	98	96	73	73	62	81	79	83	88	97	84
13	68	63	61	64	65	69	83	81	58	58	58	65	59	62	67	76	66
14	76	79	74	88	78	69	89	92	70	73	66	72	68	68	74	74	76
15	69	69	66	66	68	71	86	81	59	63	63	65	59	62	67	78	68
16	72	78	73	71	79	78	85	-	61	64	59	65	61	69	87	94	73
17	81	83	82	85	88	87	93	-	72	77	76	77	80	81	83	85	82
18	76	79	76	88	76	76	86	94	71	70	62	71	68	63	77	77	76
Mean	70	72	68	71	69	69	82	81	63	66	67	66	63	64	69	75	70
S.E±	1.3	1.2	1.4	2.4	2.1	2.2	3.6	2.8	1.5	3.0	3.7	0.7	0.9	1.1	1.6	2.7	
LSD (5%)	3.7	3.3	3.9	6.8	6.0	6.3	10.4	8.2	4.2	8.6	10.7	2.0	2.6	3.1	4.7	7.8	
Repeatability	0.90	0.87	0.73	0.79	0.93	0.97	0.95	0.89	0.97	0.96	0.93	0.81	0.94	0.83	0.52	0.98	0.87
Correlation (GY x DtF[††])	-0.67*	-0.62*	-0.81**	-0.44	-0.80**	-0.80**	-0.88**	-0.69*	-0.33	-0.77**	-0.49*	-0.46	-0.74**	-0.65*	0.85**	-0.54*	

[†] See Table 1 for genotype codes, [††] Days to flowering; *, ** significant at P < 0.05, P < 0.01probability level, respectively.

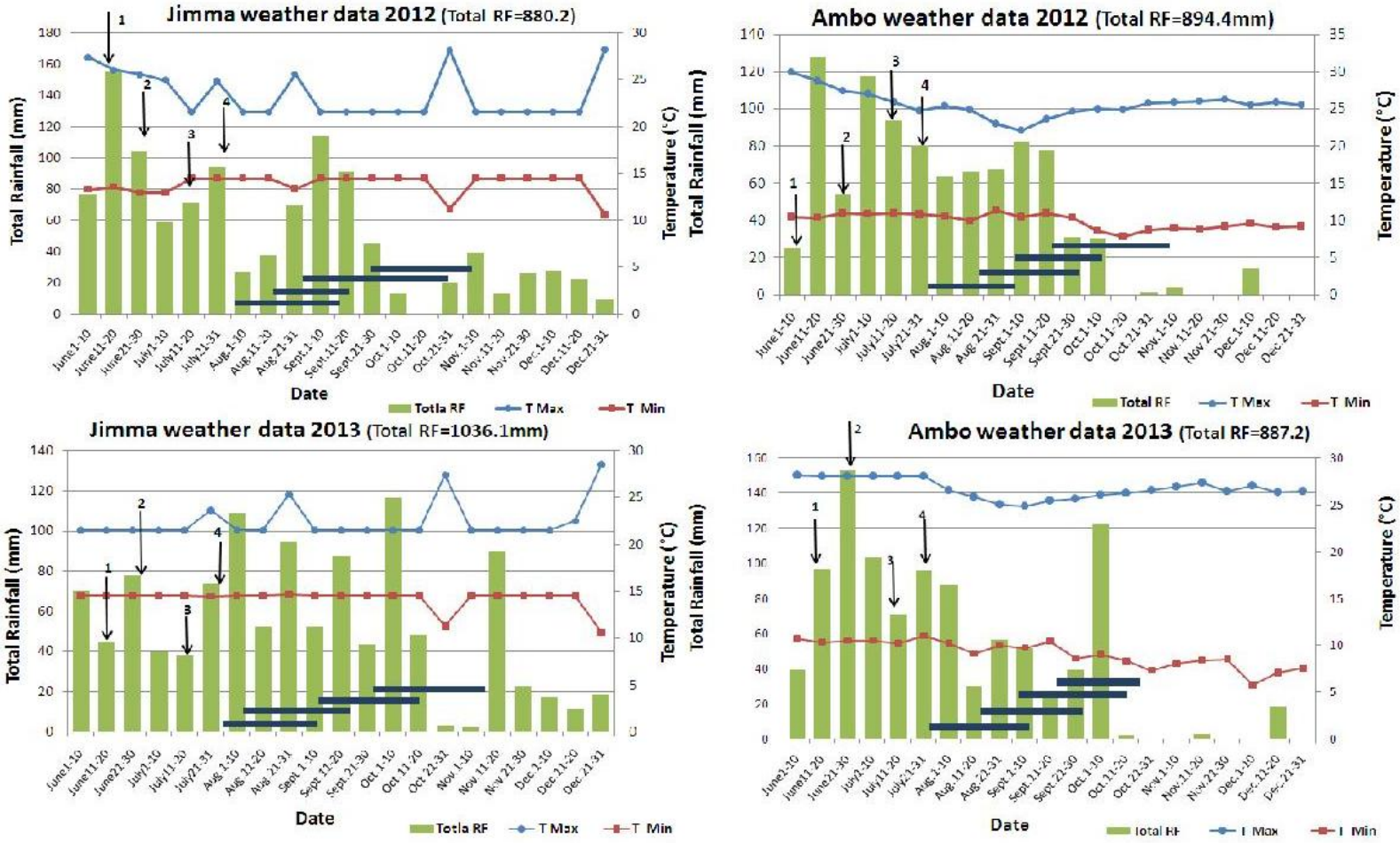

Figure S1. Total rainfall, minimum and maximum temperature of the study areas in 2012 and 2013 crop season. The four sowing dates are indicated with arrows and the black horizontal lines represent the time taken to 50% flowering from bottom to top in order of 1st, 2nd, 3rd and 4th sowing dates.