Yield stability and Genotype x Environment interactions in rice (Oryza sativa L.) genotypes in northwestern Ethiopia

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Abstract

Rice is a recent introduction in Ethiopia. However, recognizing its importance as a food security crop, source of income and job opportunities, the government of Ethiopia has named it the "millennium crop" and has ranked it among the priority commodities of the country. Variety development is one of the key research components to bring sustainable production. Accordingly, 16 rainfed lowland rice genotypes were evaluated at three locations of eight environments in northwestern Ethiopia from 2006 to 2008 to identify stable and high yielding genotypes. The experiment was conducted using randomized complete block design with three replications. Combined analysis of variance showed highly significant differences among genotypes, environments and genotype by environment interactions for grain yield. The additive main effects and multiplicative interaction (AMMI) analysis of variance indicated that the genotype by environment interaction (GEI) sum of squares was about 3.5 times larger than that for genotypes, which determined substantial differences in genotypic response across environments. The presence of GEI was clearly demonstrated by the AMMI model, when the interaction was partitioned among the first four interaction principal component axis (IPCA) which cumulatively captured 91.13% of the total GEI. The stability study indicated that among GEN13, GEN12, GEN10 and GEN9, no variety was found to be stable. In this study, environments fell in to three sections where most of the tested genotypes showed specificity. Among the tested genotypes, the highest grain yield was obtained from GEN13, GEN12 and GEN9, respectively across environments. These genotypes were selected and verified, of which GEN9 has been officially released for large scale production with the breeder name "EDGET".

Key words: AMMI, GEI, rice, stability.

Introduction

Among the target commodities that have received due attention in promotion of agricultural production, rice is considered as the "millennium crop" expected to contribute in ensuring food security in Ethiopia (MoARD, 2010). Though introduced recently, the importance of rice is being recognized well both by the Government and different stakeholders as the crop is treated as one of the major national research projects, the trend of area coverage and total

production is on the increase, the number of smallscale farmers and private investors involving in production and processing and the request for improved rice varieties is increasing.

Variety development is one of the major research focuses of the national rice research project. The general rice breeding scheme includes evaluating a number of genotypes at various stages and testing selected ones at several locations. The multi-locational testing however, usually results in genotype-by-environment (GxE) interactions that often complicate the interpretation of results obtained and reduce efficiency in selecting the best genotypes (Mosavi *et al.*, 2013).

Information on genotype x environment interaction leads to successful evaluation of stable genotype , which could be used for general cultivation. Yield is a complex quantitative character and is greatly influenced by environmental fluctuations; hence, the selection for superior genotypes based on yield per se at a single location in a year may not be very effective (Sheathe *et al.*, 2012). Thus , evaluation of genotypes for stability of performance under varying environmental conditions for yield has become an essential part of any breeding program.

Several methods have been proposed to analyze genotype x environment interactions and phynotypic stability. These methods can be divided in to two major groups: univariate and multivariate stability statistics. A combined analysis of variance can quantify the interactions and describe the main effects. However, it is uninformative for explaining GEI. Among multivariate methods, the additive main effect and multiplicative interaction analysis (AMMI) has been extensively applied in the statistical analysis of multi-environment cultivar trials (Gauch and Zobel, 1997; Sanni *et al.*, 2009; Nassir and Ariyo, 2011).

The AMMI model is a hybrid that involves both additive and multiplicative components of the two-way data structure. AMMI biplot analysis is considered to be an effective tool to diagnose GEI patterns graphically. In AMMI the additive portion is separated from interaction by analysis of variance (ANOVA). Then the principal component analysis (PCA), which provides a multiplicative model, is applied to analyze the interaction effect from the additive

ANOVA model. The biplot display of PCA scores plotted against each other provides visual inspection and interpretation of GEI components. Integrating biplot display and genotypic stability statistics enables genotypes to be grouped based on similarity of performance across diverse environments (Thillainathan and Femandez, 2001; Banik *et al.*, 2010; Davoud, 2011; Nassir and Ariyo, 2011).

This method has been shown to be effective because it captures a large portion of the G x E sum of squares, it clearly separates main and interaction effects that present agricultural researchers with different kinds of opportunities and the model provides agronomically meaningful interpretation of the data (Ebdson and Gauch, 2002). The results of AMMI analysis are useful in supporting breeding program decisions such as specific and broad adaptation and selection of environment (Gauch and Zobel, 1997). Therefore, the objectives of this study were to assess the extent of Genotype x Environment (GE) interaction for grain yield, to evaluate rice genotypes for their yield performance and stability and to select and release genotypes with high grain yield and other desirable traits either for specific and/or wide area production depending on their differential responses to environments.

Materials and methods

Fourteen rainfed lowland rice genotypes which were promoted from preliminary variety trial to national variety trial plus two checks were evaluated in northwestern Ethiopia from 2006 to 2008 at three locations of eight environments (ENV) including, Woreta (ENV1, ENV2, ENV3), Addis Zemen (ENV4, ENV5) and Pawe (ENV6, ENV7, ENV8). The locations were different in soil type, altitude, temperature and total rainfall (Table 1).

	Locations				
Agroecological character	Woreta	Pawe	Addis Zemen		
Latitude	$11^{0}58'N$	11 ⁰ 9'N	11 [°] 92'N		
Longitude	37 ⁰ 41'E	36° 3'E	37 ⁰ 7'E		
Altitude (masl)	1810	1050	1780		
Annual rainfall (mm)*	1300	1457	1032		
Mean maximum temperature $(0_C)^*$	27.9	32.75	29.96		
Mean minimum temperature $(0_C)^*$	11.5	17.17	11.31		
Soil type	Vertisol	Cambisol	Fluvisol		

Table 1. Description of experimental sites.

*Mean of three years data (2006-2008).

Randomized Complete Block Design with three replications was used. Each plot had six rows of 5 m length and spaced 0.2 m apart. Fertilizer was applied at the rate of 69/23 kg/ha of N/P₂O₅ in the form of Urea and DAP, respectively. DAP was applied all at planting while Urea was applied one third at planting, one third at tillering and the remaining one third at panicle initiation. A seed rate of 60 kg/ha was used and seeds were drilled in a row. Plantings were done following the optimal dates in each respective location. Data on grain yield and some other traits were collected. However, this paper mainly focuses on grain yield data (at 14% moisture level and estimated on the basis of four central harvestable rows). Analysis of variance was done for each environment. Bartlett's test was used to assess homogeneity of error variances prior to combined analysis over environments. The grain yield data for 16 genotypes in 8 environments were subjected to be combined and AMMI analysis of variance using CropStat version 6.1 statistical software. (CropStat, 2007). In the analysis, each combination of a single location and year was considered as an environment.

AMMI uses ordinary ANOVA to analyze the main effects (additive part) and PCA to analyze the non additive residual left over by the ANOVA (Crossa, 1990). The interaction is the genotype PCA score multiplied by that of the environment. When a genotype and environment have the same sign on their respective first PCA axis, their interaction is positive, if different, their interaction is negative. An AMMI plot is a graph where aspects of both genotypes and environments are plotted on the same axis so that interrelationship can be visualized. It provides a pictorial view of the transformed G x E interaction (Crossa, 1990) for any interpretation. In a biplot where the first interaction principal component axis (IPCA1) is on the vertical axis and mean yield on the horizontal, genotypes that appear almost on a perpendicular line had similar means and those that fall almost on a horizontal line had similar interaction patterns. Genotypes or environments with large IPCA1 scores, either positive or negative had large interactions, where as genotypes with IPCA1 score of zero or nearly zero had smaller interactions (Crossa, 1990). The biplot of the first two IPCA axes demonstrates the relative magnitude of the GEI for specific genotypes and environments. The further away from the axes center genotype or environment is, the larger the GEI.

Results and discussion

Analysis of variance

The analysis of variance for grain yield indicated that there were significant differences among the tested genotypes in each respective environment (Table 2). Bartlett's test indicated homogenous error variance for grain yield in each of eight environments and allowed to proceed further for pooled analysis and the combined analysis of variance is presented in Table 3.

Genotype (G), Environment (E) and Genotype x Environment (GxE) were significant ($P \le 0.01$) for grain yield. Such statistical interaction resulted from the changes in the relative ranking of the genotypes from one environment to another. The significant GxE effects demonstrated that genotypes responded differently to the variation in environmental conditions of location which indicated the necessity of testing rice varieties at multiple locations. This also shows the difficulties encountered by breeders in selecting new varieties for release.

	Genotype	ENV1	ENV2	ENV3	ENV4	ENV5	ENV6	ENV7	ENV8	Mean
Genotypes	code	W-2006	W-2007	W-2008	AZ-2007	AZ-2008	P-2006	P-2007	P-2008	
TOX3449-117-3-3-3	GEN1	4.83 ^{abcd}	1.43 ^e	3.16d ^e	1.38 ^f	2.34 ^{de}	2.56 ^{cd}	3.89e	2.88 ^{abc}	2.81
TOX4339-WAT-44-3-3-1-2-1	GEN2	2.26 ^{fg}	2.6cd ^e	2.00 ^e	1.27^{f}	1.43 ^e	2.06 ^{de}	1.51^{bcd}	2.45^{abcd}	1.96
HOO4-7-1-B5	GEN3	2.93 ^{efg}	1.41 ^e	5.21 ^{ab}	2.18 ^{abcde}	3.75 ^{abc}	3.35 ^{abcd}	2.67^{abcd}	2.95 ^{abc}	3.06
HO13-5-3-B4	GEN4	4.50 ^{bcde}	2.01d ^e	3.96 ^{bcd}	1.84 ^{abcdef}	2.56 ^{cde}	3.52 ^{abcd}	2.17^{abcd}	2.03 ^{bcd}	2.83
SIK273-388-2-1-2	GEN5	5.75 ^{ab}	3.43 ^{dc}	5.53 ^a	1.90 ^{abcdef}	2.96 ^{bcd}	3.55 ^{abcd}	2.39^{abcd}	1.15 ^d	3.34
SIK295-291-4-2	GEN6	5.89 ^{dab}	3.13 ^{dc}	4.32 ^{abcd}	1.41 ^{ef}	2.38 ^{de}	4.95 ^a	2.26^{abcd}	2.99 ^{abc}	3.42
FOFIFA3737	GEN7	3.42^{defg}	5.25 ^{ab}	3.58 ^{cd}	1.65 ^{cdef}	1.67 ^e	4.09^{abc}	2.22 ^{abcd}	2.35 ^{abcd}	3.03
FOFIFA3730	GEN8	3.41^{defg}	5.24 ^{ab}	3.62 ^{bcd}	1.26^{f}	2.69 ^{cde}	3.49 ^{abcd}	1.37 ^{cd}	2.73 ^{abcd}	3.01
WAB189-B-B-B-8-HB	GEN9	4.82 ^{abcd}	6.60 ^a	5.67 ^a	1.80 ^{bcdef}	2.14 ^{de}	3.76 ^{abc}	2.21^{abcd}	2.47 ^{abcd}	3.69
IAC164 (Check)	GEN10	6.48 ^a	5.73 ^{ab}	5.64 ^a	1.88 ^{abcdef}	4.05 ^{ab}	0.69 ^e	0.97 ^d	3.66 ^{ab}	3.63
TGR42	GEN11	2.13 ^g	4.02 ^{bc}	4.97 ^{abc}	1.47 ^{def}	3.17 ^{abcd}	2.96 ^{bcd}	2.97 ^{abc}	3.04 ^{abc}	3.09
AD03	GEN12	3.02 ^{efg}	5.34 ^{ab}	5.29 ^{ab}	2.42 ^{abc}	4.31 ^a	4.39 ^{ab}	3.02 ^{abc}	3.88 ^a	3.96
AURAT17	GEN13	4.02^{cdef}	5.41 ^{ab}	5.07 ^{abc}	2.49 ^{ab}	3.74 ^{abc}	4.49 ^{ab}	3.46 ^{ab}	3.83 ^a	4.07
AURAT05	GEN14	2.79 ^{efg}	5.25 ^{ab}	4.33 ^{abcd}	2.62 ^a	4.02 ^{ab}	3.84 ^{abc}	2.69 ^{abcd}	2.87^{abc}	3.55
AURAT7	GEN15	2.98 ^{efg}	5.56 ^{ab}	4.49 ^{abc}	2.53 ^{ab}	3.27 ^{abcd}	3.67 ^{abc}	3.06 ^{abc}	1.40 ^{cd}	3.40
XJIGNA(check)	GEN16	5.64 ^{abc}	6.32 ^a	4.52 ^{abcd}	2.22 ^{abcd}	2.32 ^{de}	2.52 ^{cd}	0.86 ^d	1.90 ^{cd}	3.29
MEAN		4.05	4.30	4.47	1.89	2.92	3.37	3.36	2.60	
CV (%)		22	21.5	17	21.6	22	24.4	32	31.7	
F-test :		* **	* **	* **	* **	* **	* **	*,NS	*,NS	
5%1%										

Table 2. Grain yield (t/ha) of lowland rice genotypes tested at 3 locations (Woreta, Addis Zemen and Pawe) of 8 environments from 2006 to 2008.

*, ** indicate significance at $P \le 0.05$ and $P \le 0.01$, respectively. ENV = Environment, GEN = Genotype, W = Woreta, AZ = Addis Zemen, P = Pawe, NS = non significant.

Source of	Degree of	Sum of	Mean	Explained SS
variation	freedom	square	square	(%)
Total	383	888.278		
Replications	2	0.147		
Environment (E)	7	302.819	43.259**	34.09
Genotypes(G)	15	93.441	6.229**	10.52
G*E	105	309.091	2.943**	34.78
Error	254	182.850	0.719	

Table 3. Combined analysis of variance of grain yield for 16 lowland rice genotypes evaluated at eight environments in 2006-2008.

** Significant at P≤0.01.

The factors explained (%) show that rice grain yield was affected by environment (34.09%), genotype (10.52%) and their interaction (34.78%). The mean grain yield of the 16 genotypes ranged from 1.96 to 4.07 t/ha. And the highest grain yield was obtained from genotypes, GEN13, GEN12 and GEN9 (Table 4).

AMMI analysis

The AMMI analysis of variance for grain yield of 16 genotypes tested in eight environments showed that 42.02% of the total sum of squares was attributed to environmental effects, only 12.9% to genotypic effects and 45.19% to GxE interaction effects (Table 3). As indicated in Table 1, the testing locations and/or environments were diverse and caused the greatest variation in grain yield which is in agreement with the findings by Sanni *et al.* (2008), Nassir and Ariyo, (2011) and Sadeghi *et al.* (2011). This indicated the overwhelming influence that the environments have on the performance of genotypes.

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		Grain		Plant	% filled	Thousand	Disease	e score (0-9)
	Genotype	yield	Days to	height	spikelets/	seed		X
Genotype	code	(t/ha)	maturity	(cm)	panicle	Weight (g)	LB	PB
TOX3449-117-3-3-3	GEN1	2.81 ^f	143.0 ^{ab}	92.1 ^{bcde}	89.3 ^{cd}	29.6 ^{bc}	1.3	1.5
TOX4339-WAT-44-3-3-1-2-1	GEN2	1.96 ^g	141.6 ^{bc}	89.0 ^{def}	88.7 ^{cde}	27.7 ^{de}	1.5	1.6
HOO4-7-1-B5	GEN3	3.06 ^{def}	141.4 ^{bc}	97.6 ^b	85.4 ^{de}	27.0 ^{ef}	1.5	1.9
НО13-5-3-В4	GEN4	2.83 ^f	143.0 ^{ab}	89.6 ^{def}	90.2 ^{bc}	30.5 ^b	1.6	2.0
SIK273-388-2-1-2	GEN5	3.34^{abcd}	139.1°	87.3 ^{ef}	91.4 ^{bc}	27.6 ^{de}	1.8	1.4
SIK295-291-4-2	GEN6	3.42^{bcde}	145.0 ^a	85.7^{f}	84.3 ^{de}	29.0 ^{cd}	1.2	1.3
FOFIFA3737	GEN7	3.03 ^{def}	130.0 ^{ef}	94.0 ^{bcd}	92.4 ^{abc}	30.9 ^b	1.5	1.4
FOFIFA3730	GEN8	3.01 ^{ef}	130.5 ^e	88.5 ^{def}	93.1 ^{abc}	30.4 ^b	1.6	1.3
WAB189-B-B-B-8-HB	GEN9	3.69 ^{abc}	127.7 ^f	87.8 ^{ef}	96.8 ^a	32.3 ^a	1.0	1.0
IAC164 (Check)	GEN10	3.63 ^{abc}	135.9 ^d	90.5 ^{cdef}	92.1 ^{abc}	23.8 ^g	2.0	2.4
TGR42	GEN11	3.09 ^{def}	132.0 ^e	94.1 ^{bcd}	89.2 ^{cd}	28.2 ^{cde}	1.8	2.0
AD03	GEN12	3.96 ^{ab}	133.1 ^e	96.1 ^{bc}	93.7 ^{abc}	27.8 ^{de}	2	2.5
AURAT17	GEN13	4.07 ^a	132.1 ^e	105.0 ^a	92.1 ^{abc}	28.3 ^{cde}	1.5	1.5
AURAT05	GEN14	3.55 ^{abcde}	131.6 ^e	103.4 ^a	93.5 ^{abc}	28.1 ^{de}	2.1	2.5
AURAT7	GEN15	3.40^{abcde}	131.6 ^e	97.5 ^b	92.4 ^{abc}	27.7 ^{de}	1.8	2.4
XJIGNA(check)	GEN16	3.29 ^{cdef}	130.9 ^e	96.3 ^{bc}	94.3 ^{ab}	25.8^{f}	2.0	2.0
MEAN		3.26	135.5	93.4	91.2	28.4	1.6	1.9
CV(%)		25.6	3.5	10.1	6.3	8.4		
F-test(5%, 1%):								
Genotype(Gen)		* **	*,**	* **	* **	* **		
Environment		* **	*,**	* **	* **	NS		
(Env)								
Gen *Env		* **	* **	* **	* **	*		

Table 4. Grain yield and some other agronomic traits of lowland rice genotypes tested in eight environments from 2006 to 2008.

Gen *Env *,** *,** *,** *,** * Means followed by the same letter with in column are not significantly different NS = Not significant, *, ** indicate significance at $P \le 0.05$ and $P \le 0.01$, respectively; LB = Leaf blast, PB = Panicle blast, ENV = Environment, GEN = Genotype. Sanni *et al.* (2009), Banik *et al.* (2010), Nassir and Ariyo (2011), and Hassanpanah (2011) also reported similar results that all the genotypes, environments and genotype x environment effects were declared significant in the ANOVA of AMMI. The GxE sum of squares was about 3.5 times larger than that for genotypes, which determined substantial differences in genotypic response across environments.

The presence of GEI was clearly demonstrated by the AMMI model, when the interaction was partitioned among the first four interaction principal component axis (IPCA) as they were significant P = 0.01 in a post assessment. The IPCA1 explained 39.01% of the interaction sum of squares in 21% of the interaction degree of freedom (DF). Similarly, the second, third and fourth principal component axis (IPCA 2-4) explained a further 29.27%, 14.62% and 8.63% of the GEI sum of square, respectively (Table 5).

Source	DF	SS	MS	Explained SS (%)
Genotype(G)	15	30.94	2.06**	12.79
Environment(E)	7	101.63	14.52**	42.02
G*E	105	109.28	1.04**	45.19
IPCA1	21	42.64	2.03**	39.01
IPCA2	19	31.99	1.68**	29.27
IPCA3	17	15.99	0.94**	14.62
IPCA4	15	9.43	0.63*	8.63
G*E residual	33	9.23	0.27	
Total	127	241.85		

Table 5. Additive Main effects and Multiplicative Interaction (AMMI) analysis of variance for grain yield of 16 lowland rice genotypes across eight environments.

*, ** Significant at P \leq 0.05 and P \leq 0.01, respectively.

They cumulatively captured 91.13% of the total GEI using 72 DF. This implied that the interaction of the 16 rice genotypes with eight environments was predicted by the first four components of genotypes and environments which is in agreement with the recommendation

of Sivapalan *et al.* (2000). However; this contradicted the findings of Gauch and Zobel (1997) which recommended that the most accurate model for AMMI can be predicted using the first two IPCAs. These results indicate that the number of terms to be included in an AMMI model cannot be specified a prior without first trying AMMI predictive assessment (Kaya *et al.*, 2002). In general, factors like type of crop, diversity of the germplasm and range of environmental conditions will affect the degree of complexity of the best predictive model (Crossa, 1990; Muthuramu *et al.*, 2011).

The AMMI analysis provided a biplot (Fig 1) of main effects and the first principal components (IPCA1) of both genotypes and environments. The differences among genotypes in terms of direction and magnitude along the X-axis (yield) and Y-axis (IPCA1 scores) are important. In the biplot display, genotypes or environments that appear almost on a perpendicular line of a graph had similar mean yields and those that fall almost on a horizontal line had similar interactions (Crossa, 1990). Thus the relative variability due to environments was greater than that due to genotypic differences. Genotypes or environments on the right side of the mid point of the perpendicular line have higher yields than those on the left side. As a result, genotypes including GEN13, GEN12, GEN9, GEN10, GEN14 and GEN6 were generally high yielding (4.07, 3.96, 3.69, 3.64, 3.55 and 3.42 t/ha, respectively (Fig 1). In contrast genotypes including GEN1, GEN2 and GEN4 were generally low yielding genotypes. Environments including ENV1, ENV2, ENV3 and to some extent ENV6 were always on the right hand side of the mid point of the main effect axis ,seemed to be favorable environments, while ENV4 and ENV5 were generally less favorable environments.

Genotypes or environments with large negative or positive IPCA scores have high interactions, while those with IPCA1 scores near zero (close to horizontal line) have little interaction across environments and vice versa for environments (Crossa, 1990) and are considered more stable than those further away from the line. In the biplot, genotypes including GEN13, GEN12, GEN10 and GEN9 were vertically distant apart; however, they did not fall close to the horizontal line. This implies that these genotypes lack stability but had high yield potential in favorable environments.



Mean grain yield (t/ha)

Fig 1. AMMI biplot of 16 rice genotypes and eight environments for grain yield (t ha⁻¹) using genotypic and environmental scores.

GEN1 = TOX3449-117-3-3-3, GEN2 = TOX4339-WAT-44-3-3-1-2-1, GEN3 = HOO4-7-1-B5, GEN4 = HO13-5-3-B4, GEN5 = SIK273-388-2-1-2, GEN6 = SIK295-291-4-2, GEN7= FOFIFA3737, GEN8 = FOFIFA3730, GEN9 = WAB189-B-B-B-B-HB, GEN10 = GEN10 = IAC164, GEN11 = TGR42, GEN12 = AD03, GEN13 = AURAT17, GEN14 = AURAT05, GEN15 = AURAT 7, GEN16 = X-Jigna ENV1 = Woreta06, ENV2 = Woreta07, ENV3 = Woreta08, ENV4 = AddisZemen07, ENV5 = Addis Zemen08, ENV6 = Pawe06, ENV7 = Pawe07, ENV8 = Pawe.

Since, IPCA2 scores were also important (29.27% of G x E SS) in explaining GEI, the biplot of the first two IPCAs was also used to demonstrate the relative magnitude of the GEI for specific genotypes and environments (Fig 2). The IPCA scores of genotypes in the AMMI analysis is an indication of stability or adaptation over environments (Gauch and Zobel, 1997). The greater the IPCA scores, the more specifically adapted is a genotype to certain environments Sanni *et al.* (2009). The more the IPCA scores approximate to zero, the more

stable or adapted the genotype is over all the environments sampled. The biplot of the first two IPCA didn't show the best adapted genotype and/or genotypes to most environments. However; GEN13, and GEN12 were well adapted to high yielding environment, ENV6 while GEN9 and GEN15 were well adapted to high yielding environment, ENV2. The varieties used as check (GEN10 and GEN16) were found to be well adapted to the high yielding environments of ENV1 and ENV3).



Fig. 2. Biplot of the second interaction principal component axis (IPCA2) against the first interaction principal component axis (IPCA1) scores for grain yield of 16 lowland rice genotypes in eight environments.

1-B5, GEN4 = HO13-5-3-B4, GEN5 = SIK273-388-2-1-2, GEN6 = SIK295-291-4-2, GEN7 = FOFIFA3737, GEN8 = FOFIFA3730, GEN9 = WAB189-B-B-B-B-HB, EN10 = GEN10 = IAC164, GEN11=TGR42, GEN12 = AD03,GEN13 = AURAT17, GEN14 = AURAT05, GEN15 = AURAT 7, GEN16 = X-Jigna ENV1 =Woreta06, ENV2 = Woreta07, ENV3 = Woreta08, ENV4 = AddisZemen07, ENV5 = Addis Zemen08, ENV6 = Pawe06, ENV7 = Pawe07, ENV8 = Pawe08.

In Fig 2, the environments fell in to three sections: the best genotypes with respect to ENV1, ENV2 and ENV3 (Woreta) were GEN10, GEN16, GEN9, GEN7, GEN8 and GEN15-

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