ANALYSIS OF GENOTYPE-ENVIRONMENT INTERACTIONS OF SILAGE MAIZE CULTIVARS UNDER ENVIRONMENTAL TRIALS

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Abstract

To evaluate the adaptability and stability of silage maize cultivars and identify the representativeness and discrimination of each testing site, a two-year field research in a randomized complete block design (RCBD) with three replicates at 10 testing sites was conducted. An additive main effect and multiplicative interaction (AMMI) model and a genotype plus genotype environment interactions (GEI) biplot (referred to as GGE hereafter) were used to analyze the data. The two-year test revealed that four cultivars (Zhongdi 175 (ZD175), Qiushuo 008 (Q008), Hengyu 1587 (H1587), and Yayuqingzhu 8 (Y8) exhibited high yield and good stability, whereas two cultivars (Zhongbeiqingzhu 410 (Z410) and Fangyu 36 (F36) had low yield and poor stability. The comprehensive application of the AMMI model and the GGE biplot could accurately and intuitively evaluate the high yield, stability, and adaptability of each cultivar.

Introduction

Maize (*Zea mays* L.) is China's largest food crop. It is also an important feed and economic and bioenergy-producing crop. With the increasing demand for maize as food, feed, and biofuel, maize quality and yield should be improved (Yue *et al*. 2018). Silage maize is harvested from the whole green plant of corn and used as feed for cattle, sheep, and other herbivorous animals after chopping and lactic acid fermentation (Kusvuran *et al*. 2015). Silage maize is the most important component of ruminant forage and widely used in animal feed. Silage maize is favored in western countries because of its high nutritional value and biological yield. Approximately 15 million cows in China need more than 3 million ha of silage maize. However, the planting area of high-quality silage maize is still insufficient (Wang *et al*. 2016). The adjustment of the structure of China's planting industry has changed the dual structure of grains and cash crops and gradually transformed into a ternary structure for the coordinated development of grain crops, forage, and economic crops. Silage maize is also used as the main feed for animal husbandry. Thus, silage maize sources play an important role in these sectors.

High and stable yields have been the primary concern of breeders (Mohammed *et al.* 2016). Yield is a complex genetic trait controlled by multiple genes, which are influenced by genotype, environment, and genotype-by-environment interaction (GEI). To screen cultivars with high and stable yields, breeders should subject new cultivars to multi-environment trials. In these trials, the high yield, stable yield, resistance, and adaptability of cultivars are assessed, and a scientific basis for cultivar promotion and utilization is provided (Caproni *et al*. 2018). Genotypes are placed in different environments to observe their performance. GEI is analyzed, and cultivars that are beneficial to production and social needs are selected. Thus, appropriate and accurate analysis methods should be used (Yan and Holland 2010).

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Statistical methods have been developed to study GEI for cultivar stability analysis in multi-environment trials (Becker and Léon 1988). Among these methods, GEI analysis involves an additive main effect and multiplicative interaction (AMMI) model, which was first proposed by Guach. This model combines principal component (PC) analysis and variance analysis to add product - form interactions to conventional genotype and environment additive models. This model not only analyzes the significance of interactions but also estimates the characteristics and forms of interactions. It has also been widely utilized for the GEI analysis of different crops. Genotype plus genotype and environment (GGE) biplot is a new method to study GEI, crop yield stability, and plot representation. Gabriel (1971) first proposed the biplot concept. Yan (2001) subsequently developed a graphical method called a GGE biplot that can analyze multi-environment trials. This biplot consists of the first principal component axis (PC1) and the second principal component axis (PC2) formed by the single-value decomposition of genotype and GEI and by the GGE double labeling scheme. A GGE double plot method is more intuitive and clearer than other analysis methods in terms of identifying stable yields, regional adaptability of cultivars, and resolving ability of a test environment for cultivars. This method also provides a basis for screening ideal cultivars, ideal plots, and utilization value and planting layout of various cultivars (Xu *et al*. 2014). AMMI models and GGE biplots are successful methods used to analyze species and environment interactions. Their analysis of GEI is similar (Rad *et al*. 2013, Mortazavian *et al*. 2014). In this study, an AMMI model and a GGE biplot were used to analyze genotype and environment interaction modes for silage maize cultivars from 2015 to 2016 in multi-environment trials to comprehensively evaluate the stability of the silage maize cultivars and the representativeness of the testing sites. This study aimed to provide a theoretical basis for further use and promotion of new silage cultivars in production.

Materials and Methods

The silage maize (*Zea mays* L.) genotypes used in this study and their supplier organizations and institutions are provided in Table 1. Twenty three silage maize cultivars were grown in muti-environment trials throughout two crop seasons in 2015 - 2016. Their basic information is also presented in Table 1. Experiments were conducted across 10 environments, namely Dezhou (DZ), Gaoyang (GY), Jinghai (JH), Handan (HD), Liaocheng (LC), Linfen (LF), Luoyang (LY), Suzhou (SZ), Yuncheng (YC) and Zhengzhou (ZZ). The geographic and weather characteristics of the testing sites are shown in Table 2.

The experimental design RCBD was used in this study with three replicates. The experimental plots were 6.7 m in length and 3.0 m in width. Each plot had five rows with a row-to-row distance of 60.0 cm and a planting density of 75,000 plants/ha. Sowing dates ranged from 15 June to 20 June at each testing site depending on the harvest time of wheat. Before sowing was conducted, 150 kg N/ha, 70 kg P_2O_5/ha, and 70 kg K_2O/ha were added to the soil. Weeding work was carried out twice, that is, before the seedlings sprouted and at the three-leaf stages. All of the silage maize cultivars were harvested at the milk-dough stage, and three lines in the middle of each plot were harvested manually. All of the agricultural operations during the trial were based on local field production.

ANOVA was conducted to detect the differences between cultivars, environments, and cultivars and environments. Microsoft Excel version 2007 (Microsoft Corporation) was used for data processing. GEI was examined using the AMMI model and the GGE biplot embedded in GenStat (Genstat, 18th edition):

(1) GGE model: $Y_{ij} - \mu - \beta_j = a_i + j_{ij}$ ...

(2) AMMI model: $Y_{ij} - \mu - \beta_j - a_i = j_{ij}$ ...

where Y_{ij} represents the measured mean of the ith genotype in the jth environment, μ represents the grand mean, a_i represents the main effect of the ith genotype, $β_j$ represents the main effect of the jth environment, j_{ij} represents the interaction between ith genotype and j environment (Nzuve *et al*. 2013).

Table 1. Basic information of the cultivars and its code used in experiment.

Cultivar	Code	Plant type	Breeding institute	Year
Zhaoyu 27	Z27	Flat	Hebei Zhaoyu Seed Industry Co., Ltd.	2015
Hengyu 1587	H1587	Semi-compact	Dryland Farming Institute，Hebei Academy of Agriculture and Forestry Sciences	2015-16
Yuqingzhu 23	Y23	Flat	Henan Dajingjiu Seed Industry CO., Ltd.	2015
Zhongbeiqingzhu 410	Z410	"	Northern Shanxi Seed Industry CO., Ltd.	2015
Zhongdi 175	Z175	Semi-compact	Dryland Farming Institute，Hebei Academy of Agriculture and Forestry Sciences	2015-16
Nongda 108	N108	Flat	China Agricultural University	2015
Hongruiqingzhu 101	H101	"	Hongrui Seed Industry Co., Ltd.	2015-16
Heyu 36	H36	"	Beijing Zhongnong Sanhe Agricultural Technology Co., Ltd.	2015-16
Zhaoyu 3318	Z3318	"	Hebei Zhaoyu Seed Industry Co., Ltd.	2015-16
Zhengdan 958	Z958	Semi-compact	Henan Academy of Agriculture and Forestry Sciences	2015
Wangheqingzhu 1	W1	Semi-compact	Beijing Guangyuan Wanghao Seed Industry Co., Ltd.	2015
Qingzhuxunqing 518	Q518	Flat	Xuanhua Xuntian New Technology Seed Industry Co., Ltd.	2015
Huaiyanqingzhu 6	H6	"	Beijing Wannong Seed Research Institute Co., Ltd.	2015
Qiushuo 008	Q008	"	Hebei Qiushuo Seed Industry Co., Ltd.	2015-16
Yayuqingzhu 8	Y8	"	Sichuan Yayu Technology Development Co., Ltd.	2015-16
Jiashi 14006	J14006	Semi-compact	Jiahe Seed Industry Co., Ltd.	2016
Nongfengqingzhu 166	N166	Semi-compact	Beijing Wannong Pioneer Biotechnology Co., Ltd.	2016
Qingzhuxunqing 938	Q938	Flat	Xuanhua Xuntian New Technology Seed Industry Co., Ltd.	2016
Zhuqing 1	Z1	Semi-compact	Guiyang Agricultural Machinery Promotion Center	2016
Fangyu 36	F36	Flat	Hebei Dehua Seed Industry Co., Ltd.	2016
Xianyu 1321	X1321	"	Pioneer Seed Co.	2016
Xianyu 1581	X1581	"	Pioneer Seed Co.	2016
Jinzhu 100	J100	Semi-compact	Tianjin Zhongtian Dadi Technology Co., Ltd.	2016

Table 2. Description of the testing site for the evaluation of silage maize cultivars in 2015 - 16.

Testing site	Province	Code	Longitude (E)	Latitude (N)	Altitude (m)	Annual rainfall (mm)	Mean annual temp. (℃)
Dezhou	Shandong	DZ	116°27′	37°28′	22	547	13.1
Gaoyang	Hebei	GY	115°78′	38°69′	14	312	12.5
Jinghai	Tianjin	JH	116°98′	38°95′	6	686	13.9
Handan	Hebei	HD	114°53′	36°64′	55	424	14.6
Liaocheng	Shandong	LC	115°98′	36°45′	37	543	13.6
Linfen	Shanxi	LF	115°50′	36°05′	450	478	12.2
Luoyang	Henan	LY	112°43′	34°62′	138	485	15.1
Suzhou	Anhui	SZ	116°94′	33°63′	28	825	16.2
Yuncheng	Shanxi	YC	111°01′	35°02′	369	502	14.3
Zhengzhou	Henan	ZZ	113°62′	34°73′	110	537	15.7

Results and Discussion

ANOVA showed that genotypes, environments, and GEI significantly affected the silage biomass yield in 2015 ($p < 0.01$). Environments accounted for 42.53% of the sum of squares (SS), and genotypes and GEI corresponded to 19.07 and 18.47% of the SS, respectively (Table 3). Among the three influencing factors, environments had the greatest influence on the stability of the tested cultivars, indicating that the tested cultivars significantly differed (Kendal *et al.* 2016, Amare and Adisu 2017). Therefore, different environmental conditions can be used as a basis for selecting appropriate cultivars (Abakemal *et al.* 2016). GEI plays an important role in the stability of the tested cultivars (Kandus *et al.* 2010). However, the stability of the cultivars was analyzed because the variation in environments accounted for the majority.

The regression analysis of the silage maize yields (Table 3) revealed that the combination of joint regression, genetic regression, and environmental regression accounted for 28.58% of the total SS. The residual was still large, accounting for 71.42%, and joint regression, genetic regression, environmental regression, and residual were extremely significant ($p < 0.01$). These results indicated that the regression model showed low interaction and had poor results after the experimental data were fitted.

ANOVA revealed that the effects of genotypes, environments, GEI in 2016 were significant, and SS explained 38.16% for environmental effects, 11.48% for environmental effects, and 27.31% for GEI effects. Linear regression analysis indicated that the sum of joint regression, gene regression, and environmental regression accounted for 35.19% of the interaction, and the residual accounted for 64.81%. These values reached extremely significant levels ($p < 0.01$). The AMMI model results suggested that all of the interaction principal component axes (IPCAs) accounted for 76.81% of the interaction, and the residual was 23.19% (Table 4).

The GEI effect was further divided into IPCAs, namely IPCA1, IPCA2, IPCA3, and residual effects. All IPCAs reached a significant level and accounted for 40.51, 21.67 and 13.56% of the GEI variation. The residuals corresponded to 24.25% (Table 3); that is, the total interactions of the first three items accounted for 75.74%. The AMMI model could be used to thoroughly analyze GEI interaction information, and the AMMI model was significantly better than traditional regression models. Therefore, the AMMI model could effectively overcome the limitations of linear regression analysis in evaluating the stability of plant genotypes (Oyekunle *et al.* 2017).

Table 3. Analysis of variance, linear regression analysis and AMMI model analysis in 2015.

Methods	Source of variance	Degree of freedom	Sum of squares	Mean squares	%/ total SS	%/SS of interaction	F value
AOVA	Total variance	449	20279109.69	45165.06	-	-	
	Treatment	149	16237081.48	108973.70	-	-	8.09**
	Genotype	14	3867765.72	276268.98	19.07	-	20.50**
	Environment	9	8623226.89	958136.32	42.53	-	71.11**
	Genotype and environment interaction	126	3746088.87	29730.86	18.47	-	2.21**
	Error	300	4042028.21	13473.43	19.93	-	-
Linear regression analysis	Joint regression	1	101545.73	101545.73	-	2.71	7.54**
	Genetic regression	13	651138.06	50087.54	-	17.38	3.72**
	Environmental regression	8	318101.01	39762.63	-	8.49	2.95**
	Residual	104	2675304.06	25724.08	-	71.42	1.91**
AMMI model	IPCA1	22	1517548.74	68979.49	-	40.51	5.01**
	IPCA2	20	811964.29	40598.21	-	21.67	2.95**
	IPCA3	18	508020.67	28223.37	-	13.56	2.05**
	Residual	66	908555.18	13765.99	-	24.25	

- Mean not existed, ** Mean significant at 1% probability level.

Table 4. Analysis of variance, linear regression analysis and AMMI model analysis in 2016.

Methods	Source of variance	Degree of freedom	Sum of squares	Mean squares	%/ total SS	%/SS of interaction	F value
AOVA	Total variance	449	26125271.79	58185.46	-	-	
	Treatment	149	20105696.21	134937.56	-	-	6.72**
	Genotype	14	3000971.379	214355.09	11.48	-	10.68**
	Environment	9	9969864.35	1107762.70	38.16	-	55.21**
	Genotype and environment interaction	126	7134860.48	56625.88	27.31	-	2.82**
	Error	300	6019575.58	20065.25	23.04	-	-
Linear regression analysis	Joint regression	1	3877.81	3877.81	-	0.05	0.19
	Genetic regression	13	1380416.19	106185.86	-	19.35	5.29
	Environmental regression	8	1126790.88	140848.86	-	15.79	7.01
	Residual	104	4623775.58	44459.38	-	64.81	2.21
AMMI model	IPCA1	22	2456853.42	111675.15	-	34.43	4.45
	IPCA2	20	1685117.44	84255.87	-	23.62	3.36
	IPCA3	18	1338608.91	74367.16	-	18.76	2.96
	Residual	66	1654280.69	25064.85	-	23.19	

- and ** same as in Table 3.

The cultivars located farthest from the origin in the same direction were connected to form a polygon, and all of the other cultivars were placed inside the polygon. From the origin (0,0), the sides of the polygon were perpendicular. These vertical lines cut the entire double graph into multiple sectors, thereby dividing the test points into different groups. The cultivar located in the top corner position of each sector showed the highest yield in the region in each environment. The cultivars inside the polygon and close to the origin were close to the average yield and were insensitive to environmental changes (Sharma *et al*. 2010).

The PC1 of the yield traits of silage maize in the multipoint identification trial in 2015 accounted for 56.79% of genotype + GE, whereas PC2 corresponded to 19.69% genotype + GE. The GGE double biplot could explain 76.47% of the G + GE interactions (PC1 + PC2) (Fig. 1). Z27, N108, Z410, Z958, H36 and Z175 were at the top of the polygon, respectively, indicating that these cultivars performed the best in their area. H101 had the highest yield in LF and SZ pilots. Z175 had the highest output in ZZ, DZ, LC, HD and JH. H36 had the highest yield in GY; Z958, Z410, N108, and Z27 did not have plots, thereby indicating that these cultivars did not perform well in all of the testing sites.
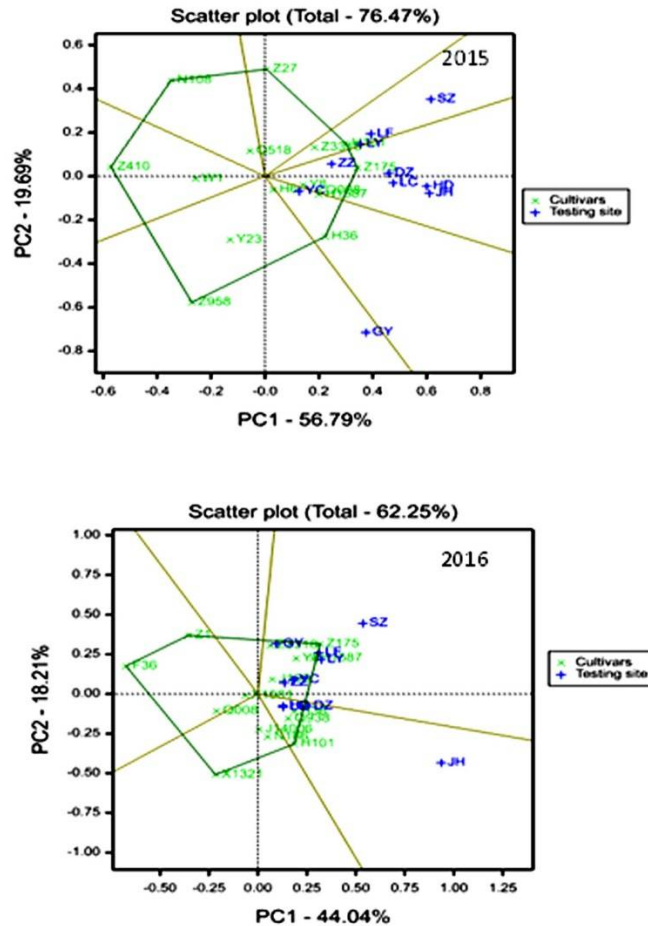

Fig. 1. Adaptability of silage maize cultivars for biomass yield based on GGE-biplot analysis.

The first two principal components (PC1 + PC2) explained 62.25% of genotype + GE in 2016 (PC1 44.04% and PC2 18.21%）(Fig. 1). Z175, H101, X1321, F36 and Z1 were located at the top of the polygon that was divided into four sectors. The plots in the first sector were SZ, GY, LF, LY, ZZ and YC. Among them, Z175 had the best performance in the first sector, thereby suggesting that Z175 had good adaptability, and the biomass yield was high in these plots. The plots in the second sector were JH, DZ, LC and HD; H101 showed the best performance in the testing sites. X1321, F36 and Z1 did not have plots in the sector, thereby indicating that these cultivars performed poorly in all the plot sites.
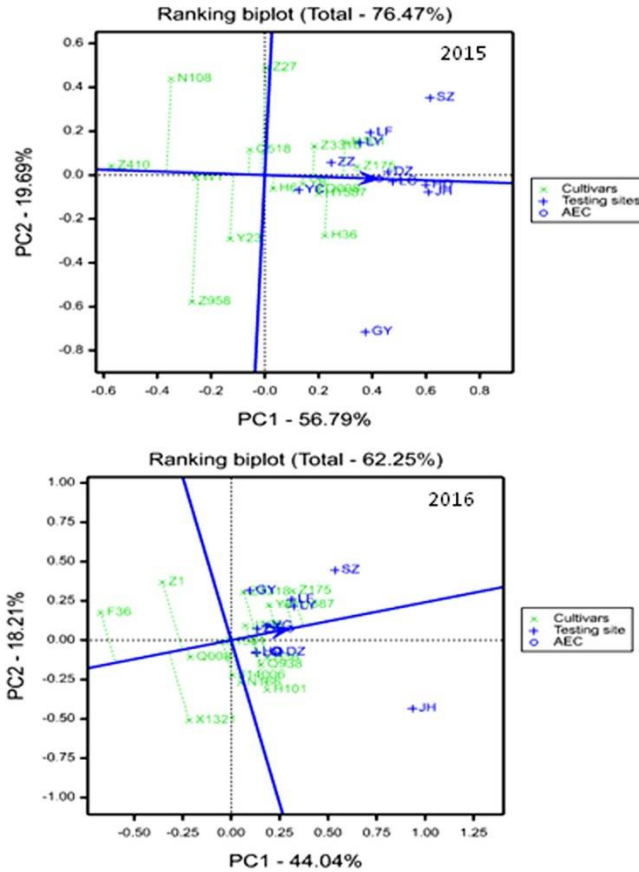
Fig. 2. Analysis of high yield and stable yield of silage maize cultivars.

The comprehensive analysis of the two-year data revealed that the performance and adaptability of Z175 and H101 were better than those of the other cultivars. The biomass yields of Z958, Z410, N108, Z27, X1321, F36 and Z1 were relatively low in all of the testing sites.

In Fig. 2, the straight line with an arrow was the average environmental coordinate (AEC) axis, the circle on the straight line was the average environmental value, and the straight line passing through the center and the average environmental axis. A vertical line was made between the cultivar point and the AEC for the analysis of the yield and stability of the tested cultivars. The direction of the AEC was the trend of the approximate average yield of the cultivar under all of the circumstances (Mehari *et al.* 2015).

In the multi-environment trial in 2015, the highest average biomass yield was observed in Z175, followed by H101, H36, H1587, Q008, Z3318, Y8, H6, Z27, Q518, Y23, Z958, W1, N108 and Z410. Passing the center (origin), the line perpendicular to the average axis represented the tendency of each cultivar to interact with each environment. The longer the vertical line between the cultivars and the AEC was, the more unstable the cultivars would be (Lakew *et al.* 2014). The most unstable cultivar was Z958, followed by Z27 and N108. The stability of Z410, Y8, Z175, H6 and H1587 was good.

In the multi-environment trial in 2016, the highest average biomass yield was found in Z175, followed by H1587, Y8 and H36. By contrast, the average yield of F36 was the lowest. In terms of the stability of cultivars, Q008, J100, Y8, H1587 and Z175 were more stable than the other cultivars. Combined with identification test for two years, Z175 and H1587 had high and stable yields. Z410, Q008, H6 and Y8 had good stability and poor biomass yield. N108, Z958, X1321, Z1 and F36 had low yield and poor stability.

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