Genetic diversity of maize lines for traits related to maturity and yield components

Élcio Friske*[1], Adilson Ricken Schuelter[1], Ivan Schuster[2], Jonatas Marcolin[2], Mayara Fabiana da Silva[2]

[1]Universidade Estadual do Oeste do Paraná, Marechal Cândido do Rondon-PR, Brazil
[2]Cooperativa Central de Pesquisa Agrícola, Cascavel-PR, Brazil

*Corresponding author: elciofriske@hotmail.com

Abstract

Grain yield and maturity class are among the commercial traits that most often direct maize breeding that aims to meet the requirements of the seed market. Thus, the aim of this study was to evaluate genetic diversity in maize inbred lines for grain maturity and yield components. The phenotypic attributes of 81 maize inbred lines were assessed in a field experiment in a square lattice design with three replications. Analysis of variance and multivariate analysis were performed considering complete randomized blocks due to the equivalence to the estimation for lattice efficiency. The results of analysis of variance indicated genetic diversity in the germplasm for all the traits, showing high variability for number of days to male flowering (DMF) and female flowering (DFF). Genetic diversity was also detected for grain water loss, which was determined by the area under the water loss curve (AUWLC), an index representing the dynamic progress of field water loss, based on area under the disease progress curve (AUDPC). The high magnitude of genetic correlation between the AUWLC and the moisture content of grain (0.98) collected at different moments in the R6 stage suggests the possibility of its substitution by the moisture content evaluation in only one collection. Weak genetic correlations (0.07 to 0.45) between yield and maturity components indicated the possibility of selection for earliness without compromising yield. In addition, the results of the Tocher method and the UPGMA were compatible, enabling classification of the germplasm and suggestion of hybrid combinations among inbreds that might have a higher heterotic effect on yield associated with earliness.

Keywords: earliness; flowering; genetic variability; grain water loss; yield, *Zea mays*.
Abbreviations: AUDPC _ area under the disease progress curve; AUWLC _ area under the water loss curve; CD _ cob diameter; CEDR _ cob diameter/ear diameter ratio; DFF _ number of days to female flowering; DMF _ number of days to male flowering; ED _ ear diameter; EGWR _ ear weight/grain weight ratio; EL _ ear length; GW _ grain weight; HW _ hectoliter weight; KRN _ kernel row number; TGW _ thousand grain weight; UPGMA _ unweighted pair group method with arithmetic mean.

Introduction

The seed maize market has changed greatly, especially in producing regions of southern Brazil, with earlier planting dates in the summer growing season and also in the second crop season. Breeding programs have thus focused on developing hybrids with high yield potential, but with different maturity rates from those already being marketed. Thus, knowledge of genetic divergence in the germplasm for traits related to grain maturity and yield components are of importance to optimize the selection of lines and advance the process of developing competitive hybrids (Dai et al., 2017).

The earliness of a maize line or hybrid is basically determined by the number of days to flowering and the grain water loss rate (Egli, 2017). Several studies have been conducted to identify genetic control of flowering; however, different magnitudes and types of gene action were observed (Colasanti and Muszynski, 2009) given the high influence of the environment and the use of different germplasm and genetic statistical methodologies.

For estimation and evaluation of maize grain maturation, the most widely used measures are flowering date and formation of the black layer. The former is used by breeding programs to estimate the cycle and the latter is useful to producers in determining when to harvest. Although they are easy to determine, neither are very reliable measures and lead to disagreements in the results obtained.

A way of evaluating water loss is estimation of its relative rate, considered accurate when estimated in several collections. Yang et al. (2010) proposed measurement of moisture content with a digital determiner and tabulation of the data using the area under the disease progress curve (AUDPC) method thus being the area under the water loss curve (AUWLC). They affirmed that the procedure is efficient and reliable in identification of genotypes with rapid water loss.

The grain water loss rate has not been adequately studied genetically, mainly because it is a very labor intensive trait that is highly influenced by environmental conditions (Sala et al., 2006; Fuzhong et al., 2008). It is commonly determined only in pre commercial and commercial hybrids, aimed at guiding technicians along with producers. This phenotypic attribute is dependent on the genetic constitution of the

genotypes, both during and after physiological maturity, revealing the importance of genetic factors (Wang et al., 2012).

However, the selection of elite lines for earliness and yield in breeding hybrids for tropical regions is hampered by the complexity of these traits and negative correlation (Hallauer et al., 2010). This requires the evaluation of yield components that are significantly related to the maturity attributes and that indirectly favor the breeding (Lu et al., 2006; Zhan et al., 2009). The identification of genotypes with early flowering and rapid water loss without reduction in yield represents one of the biggest challenges of breeding programs.

Given the above, this study was conducted to: i) evaluate the efficiency of water loss determined through the area under the water loss curve, ii) check the use of flowering for maturity classification, and iii) evaluate the occurrence of genetic variability for flowering, grain maturity, and yield components in common maize lines.

Results and Discussion

Analysis of variance

In the results of analysis of variance (Table 1), we detected significant differences ($p \leq 0.01$) between the means of maize lines, indicating the existence of genetic variability for all traits. There was high accuracy in the test, indicated by low coefficients of variation for all traits (Fritsche-Neto et al., 2012).

In relation to the estimated genetic parameters (Table 1), the heritability coefficients and the CVg/CV ratio were higher than 0.9 and 1.0, respectively, confirming the existence of genetic variability and the importance of genetic factors in determining traits related to maturity and grain yield (Hallauer et al., 2010).

Analysis of water loss and flowering

The mean values of lines for AUWLC ranged from 599.7 to 981, divided into 10 groups by the Scott-Knott method ($p \leq 0.05$) (Table 2). The CDL08 line was the most efficient in reducing grain moisture, considered the earliest among the genotypes. The lines CDL52, CDL57, and CDL72 in group "I" also stood out as early, with area ≤ 703.6. The lines classified in "A", "B", and "C" groups had greater AUWLC by exhibiting high moisture content at the beginning of collections; they were therefore considered late lines.

For DMF, we found amplitude of 19.7 days between the earliest and the latest line. By the Scott-Knott technique ($p \leq 0.05$), of the nine groups formed, the CDL57 line showed the lowest sum of DMF, which was followed by lines CDL08, CDL14, CDL22, CDL52, and CDL72. These were considered early lines, with flowering up to 63.9 days after sowing (Table 2). For DFF, the amplitude was 21.7 days among genotypes, with eight groups formed by the Scott-Knott test ($p \leq 0.05$). The CDL57 line once more stood out for earliness of flowering, followed by lines CDL08, CDL22, CDL26, CDL52, CDL72, and CDL79. They were also considered early for this trait, with average flowering ≤ 64.2 days after sowing (Table 2).

Direct analysis of the grouping by the Scott-Knott test ($p \leq 0.05$) for DMF and DFF shows high similarities of pairs and even groups of lines, which remained grouped together in both attributes (Table 2). This association was confirmed by the estimation of genetic correlation (r_g) for these traits, 0.91, considered very strong. In general, the emergence of the male inflorescence is two to four days before the exposure of the style and stigma (Campbell et al., 2014), revealing the high correlation.

A strong genetic correlation was also detected between DMF and AUWLC (r_g=0.8) and between DFF and AUWLC (r_g=0.77), similar to the correlation observed by Yang et al. (2010) Therefore, a polynomial regression was applied to verify the possibility of estimating AUWLC from information on the number of days to flowering alone.

Polynomial regression analysis indicated that equations with quadratic effects were those that best represented the AUWLC (X) by the male flowering (Y_{DMF} = _2537.5 + 84.3 X _ 0.51 X^2; R^2 = 0.648) and female flowering (Y_{DFF} = _2667.8 + 88.9 X _ 0.55 X^2; R^2 = 0.631). However, coefficients of determination associated with regression equations were of moderate magnitude, indicating the existence of other factors not explained by the model, which may result in alternating the order of the means of genotypes. In this sense, using only the number of days to flowering is not a sufficiently effective criterion for the purpose of identifying genotypes with greater or lesser loss of grain moisture, which is required by maize breeding programs.

Considering the importance of the AUWLC trait, even with its high correlation with female and male flowering, it is necessary to check the possibility of an alternative, because determining it is very labor intensive, hampering its routine application in a breeding program. In this context, we used grain moisture content values from ears collected at four day intervals (UR1 to UR7) to estimate the genetic correlation coefficients with AUWLC, DFF, and DMF. The coefficients of genetic correlation of AUWLC with grain moisture content values were, from the third collection (UR3) on, very strong (> 0.90) and reached the magnitude of r_g = 0.98 in the fifth (UR5) and sixth (UR6) collections. In UR5 and UR6, the grain moisture contents of early maturity lines were 19.7% and 17.2%, respectively, while the contents of later lines were about 35%. This high magnitude of grain moisture content suggests the feasibility of replacing AUWLC with a single collection of moisture content, facilitating the study of water loss and its applicability in the routine of maize breeding programs.

Analysis of yield components

For yield components GW and TGW, the lines evaluated were distributed in seven and eight groups, respectively, by the Scott-Knott test ($p \leq 0.05$). The mean values of lines for GW ranged from 58.8 to 144.2 g; and CDL21, CDL36, CDL46, and CDL64 showed higher yield. For TGW, values ranged from 172.0 to 389.8 g, especially CDL11, CDL63, and CDL73 (Table 3). For the HW trait, we detected variation from 158.7 to 205.4 g among the lines, which were grouped by the Scott-Knott test ($p \leq 0.05$) into two groups of 72 lines, and the remainder was distributed in three smaller groups (Table 3). These results demonstrate the low variability for HW; however, the ratio between CVg/CV (Table 1) was 1.85, indicating a higher importance of genetic factors in relation to those of the environment, enabling the suggestion of gains by breeding.

Table 1. Analysis of variance for 12 traits evaluated in the germplasm of common maize. Cascavel, PR, Brazil, 2014-2015.

		Mean squares					
SV	DF	AUWLC	DMF	DFF	GW	TGW	HW
Blocks	2	1492.44	0.10	1.66	442.94	946.59	27.58
Genotypes	80	13100.95**	36.71**	57.04**	1101.31**	4778.47**	167.18**
Residual	160	371.55	0.90	1.21	69.10	162.08	14.80
Mean		836.03	68.24	69.42	93.96	275.15	192.83
CV (%)		2.31	1.39	1.59	8.85	4.63	2.00
CVg(%)		7.79	5.06	6.21	19.74	14.26	3.70
CVg/CV		3.38	3.64	3.92	2.23	3.08	1.85
h²		0.97	0.97	0.97	0.93	0.96	0.91
		Mean squares					
SV	DF	EGWR	ED	CD	CEDR	KRN	EL
Blocks	2	0.004	3.11	1.24	0.001	0.65	2.46
Genotypes	80	0.005**	36.48**	19.56**	0.02**	8.94**	7.26**
Residual	160	0.000	1.06	0.31	0.001	0.28	0.28
Mean		0.82	43.10	27.18	0.61	14.56	13.34
CV (%)		2.49	2.38	2.06	3.89	3.64	3.25
CVg (%)		5.24	7.97	9.32	13.69	11.67	9.33
CVg/CV		2.10	3.34	4.52	3.52	3.21	2.87
h²		0.92	0.97	0.98	0.97	0.96	0.96

** significant at 1% probability by the F_test; SV = source of variation; DF = degrees of freedom; CV = coefficient of variation; CVg(%) = coefficient of genotypic variation; CVg/CV = ratio of CVg to CV; and h² = heritability.
AUWLC = area under the water loss curve; DMF = days to male flowering; DFF = days to female flowering; GW = grain weight; TGW = thousand grain weight; HW = hectoliter weight; EGWR = ear weight/grain weight ratio; ED = ear diameter; CD = cob diameter; CEDR = cob diameter/ear diameter ratio; KRN = kernel row number; and EL = ear length.

Table 2. Scott-Knott grouping (p ≤ 0.05) for the area under the water loss curve (AUWLC), days to male flowering (DMF), and days to female flowering (DFF) in 81 lines of common maize. Cascavel, PR, Brazil, 2014_2015.

	Group	A	B		C			D			E				F				G		H		I			J
	IM	981	980.9_926.3		926.2_893.0			892.9_868.4			868.3_828.8				828.7_790.4				790.3_733.4		733.3_703.7		703.6_648.9			648.8_599.7
AUWLC		60	20	58	12	19	37	9	17	24	1	2	3	5	4	6	10	13	14	15	54	79	52	57		8 (CDL)
			59	77	40	41	43	31	32	34	7	11	18	23	16	21	25	27	22	26			72			
					61	75	80	38	42	45	28	33	35	36	29	30	44	46								
								50	63	68	39	48	55	62	47	49	51	53								
								81			64	67	70	71	56	65	66	69								
											74	76	78		73											

	Group	A		B		C			D				E				F			G			H			I
	IM	78 _ 76.6		76.5 _ 74.6		74.5 _ 72.3			72.2 _ 69.3				69.2 _ 67.6				67.5 _ 66			65.9 _ 64			63.9 _ 61.3			61.2 _ 58.3
DMF		60	61	58	75	19	40	59	2	9	12	13	17	20	23	28	1	15	16	4	11	24	8	14	22	57 (CDL)
						80			29	31	32	33	34	35	37	38	18	25	27	36	21	44	56	26	30	49	53	52	79
									39	41	42	43	45	47	50	65	46	48	51	62	64	76	54	55	63	66	
									68	69	70	74	77	78	81		67						71	72	73	

	Group	A			B			C			D						E		F				G		H	
	IM	80 _ 78.3			78.2 _ 74			73.9 _ 71.3			71.2 – 69						68.9 _ 67		66.9 _ 64.3				64. 2 _ 61.3		61.2 _ 58.3	
DFF		17	59	60	19	34	37	9	10	20	1	2	3	7	13	18	25	29	12	15	16	27	4	5	6	11	14	21	8	22	26	57 (CDL)
		61	80		42	58	75	23	28	40	31	32	33	35	38	43	44	45	36	39	46	56	24	30	49	53	54	55	52	72	79	
					77	78		41	47	69	48	50	51	65	68	70	74	81	62	64	67	71	63	66	73	76						

IM = interval of means.
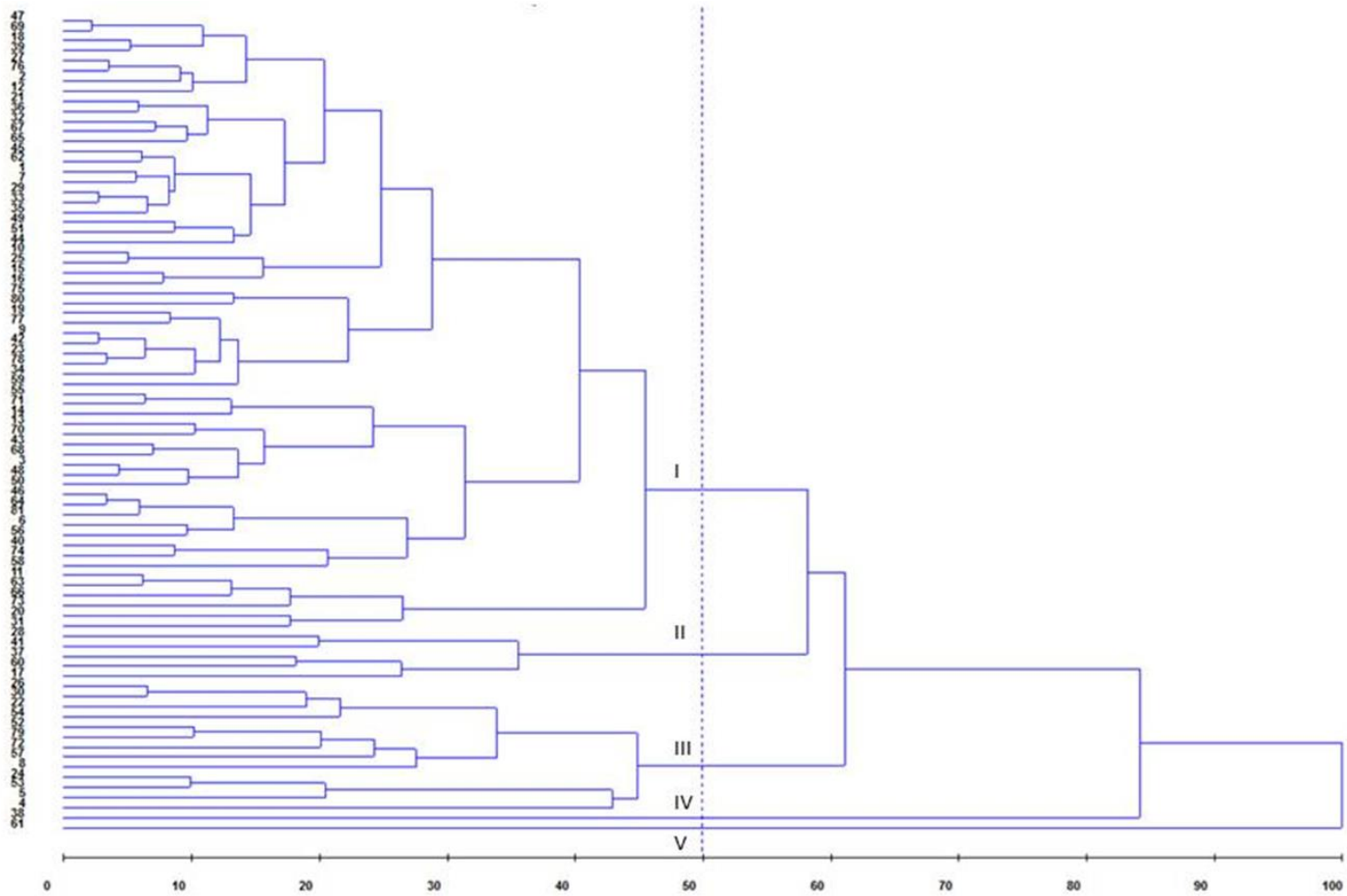
Fig 1. Dendrogram by the UPGMA based on Mahalanobis distance considering 12 genetic parameters in the germplasm of common maize. The cutoff point established at 50% classified the lines in five groups. Cascavel, PR, Brazil, 2014-2015.

Table 3. Scott-Knott grouping (p ≤ 0.05) for grain weight (GW), thousand grain weight (TGW), hectoliter weight (HW), and ear weight/grain weight ratio (EGWR) in 81 lines of common maize. Cascavel, PR, Brazil, 2014_2015.

GW

Group	A	B	C	D	E	F	G
IM	144.2 – 126	125.9 _ 112.9	112.8 _ 101.5	101.4 _ 91.8	91. 7 _ 76.7	76.6 _ 62.1	62 _ 58.8
	21 36 46	6 32 40	1 7 11 24 29	18 20 22 42	3 5 8 9	2 4 15 16	17 61 70
	64	53 65 81	31 33 34 35 37	43 45 48 51	10 12 13 14	23 25 27 38	72 80 (CDL)
			44 49 55 56 58	52 59 60 62	19 26 28 30	39 41 47 54	
			63 73 74 77	66 67 68 71	50 75 76 79	57 69 78	

TGW

Group	A	B	C	D	E	F	G	H
IM	389.8 _ 354.8	354.7 _ 330.9	330.8 _ 302.4	302.3 _ 287	286.9 _ 252.8	252.7 _ 229.1	229 _ 172.1	172.0
	11 63 73	31	12 20 21	1 2 3	18 19 22 24	6 7 10 13	5 9 14 15	8 25 26 4 (CDL)
	34	37	41	27 28 32	36 39 43 45	17 30 35 40	16 23 29 33	38 56 61
	60	64	66	46 47 48	49 50 51 52	44 54 57 72	42 55 58 62	70 75 80
	77	81		53 59 65	67 68 69 76	78	71 74 79	

HW

Group	A	B	C	D	E
IM	205.4 _ 195.2	195.1 _ 184.4	184.3 _ 172.8	172.7 _ 158.8	158.7
	1 2 3 4 7 13 15 18 19 21	5 6 8 9 10 11 12 14 16 17 22 25 27 31 37	26 30 44	20 54	38 (CDL)
	23 24 28 29 32 33 34 35 36 40	39 41 42 43 45 46 47 48 50 51 52 55 60 61 62	56 73		
	49 53 57 58 59 68 74 75 76 77	63 64 65 66 67 69 70 71 72 78 79 80 81			

EGWR

Group	A	B	C	D	E	F
IM	0.91 _ 0.86	0.85 _ 0.83	0.82 _ 0.8	0.79 _ 0.74	0.73 _ 0.66	0.65 _ 0.58
	5 24 33 35 36	1 4 6 7 11 18 20 21 22 26 27	3 9 10 12 15 16	2 8 13 14 25	61	38 (CDL)
	37 40 46 53 56	28 29 30 32 34 41 42 45 48 49 52	17 19 23 44 51 55	31 39 43 47 50		
	64 66 81	57 58 62 65 68 71 73 74 76 77 79	59 60 63 67 72 75	54 69 70 78 80		

IM = interval of means.

Table 4. Scott-Knott grouping (p ≤ 0.05) of ear diameter (ED), cob diameter (CD), cob diameter/ear diameter ratio (CEDR), kernel row number (KRN), and ear length (EL) in 81 lines of common maize. Cascavel, PR, Brazil, 2014_2015.

ED

Group	A	B	C	D	E	F	G	H
IM	51.2 _ 51	50.9 _ 48.8	48.7 _ 46.1	46 _ 43.3	43.2 _ 41.6	41.5 _ 39.7	39.6 _ 38.2	38.1 _ 35.8
	64	6 43 46	3 18 20 21 31	7 11 13 14 22 32 33	1 5 12 16 19	2 8 9 15 23	4 28 41	10 17 25
		81	36 40 48 55 56	39 44 45 49 50 51 62	26 29 30 35 38	24 27 34 37 42	75 78	57 61 72
			58 68 71 73 74	63 65 66 67 77	47 52 70 76	53 54 59 60 69		79 80 (CDL)

CD

Group	A	B	C	D	E	F	G	H	I
IM	32.3 _ 30.9	30.8 _ 29.7	29.6 _ 28.2	28.1 _ 27.1	27 _ 25.9	25.8 _ 24.8	24.7 _ 23.5	23.4 _ 22.9	22.8 _ 21.4
	6 14 48	3 43 51	13 18 20 22	2 7 9 16	1 4 11 12	8 15 34	24 25 28	5 10 57	17 37 41
	50 55 71	56 58 64	31 32 36 38	19 21 33 44	23 26 27 29	35 54 59	52 72		53 79 (CDL)
		68 81	39 40 46 49	45 47 62 67	30 42 63 69	60 66 75	80		
			61 65 70 74	73 77	76 78				

CEDR

Group	A	B	C	D	E	F	G	H	I
IM	0.84 _ 0.82	0.81 _ 0.76	0.75 _ 0.7	0.69 _ 0.67	0.66 _ 0.62	0.61 _ 0.58	0.57 _ 0.54	0.53 _ 0.5	0.49 _ 0.44
	27 40 52	58	16 18 23	5 9 22	1 6 7 8	2 11 14 26 28	4 17 19	12 15 21	3 13 42
			36 41 75	33 51 61	10 20 24 25	35 39 43 45 46	30 32 38	37 49 53	47 54 68
				65 78 79	29 31 34 44	55 57 62 63 66	48 50 56	70 74	71 (CDL)
				80	60 67 72 77	69 73 76	59 64 81		

KRN

Group	A	B	C	D	E	F	G	H	I
IM	18.7 _ 17.9	17.8 _ 16.8	16.7 _ 15.7	15.6 _ 14.9	15.6 _ 14.9	13.8 _ 13	12.9 _ 11.7	11.6 _ 11.2	11.1 _ 10.4
	6 48 74	3 40 50	13 43 44	4 9 14	1 5 7 15 17	2 8 11 16 24	10 12 28	54	41
		51 56 71	45 46 49	19 31 33	18 20 21 22 23	26 27 30 32 34	52 57 61	73	72 (CDL)
			55 62 64	35 47 58	25 29 36 38 39	37 53 60 66 67	76 79		

	81					63	68	70	42	59	65	69	78	75	77	80						
Group	A			B			C					D				E				F			G			H	
IM	19.9 _ 18.8			18.7 _ 18			17.9 _ 16.6					16.5 _ 15.9				15.8 _ 15.1				15 _ 14			13.9 _ 13.3			13.2 _ 12.5	
	19 28 59			9 32 41			1 6 7 21 23					2 11 12 20				5 8 10 17				4 13 15			3 14 43			54 57 (CDL)	
	61 65			42 53 67			24 34 36 40 45					26 27 30 35				18 25 29 31				16 22 38							
				77 78			46 51 56 58 62					37 39 44 47				33 50 55 63				48 68 70							
EL							64 75 80 81					49 52 60 76				66 69 73 74				71 72 79							

IM = interval of means.

Table 5. Tocher grouping based on Mahalanobis distance between the germplasm of common maize for 12 genetic parameters. Cascavel, PR, Brazil, 2014_2015.

Groups	Lines													
I	47	69	27	1	23	34	78	7	9	42	29	35	45	62	33
	18	67	39	77	32	76	2	16	36	49	51	21	65	30	44
	15	46	81	31	13	68	50	70	12	74	19	40	25	56	66
	63	24	10	26	3	43	64	58	20	6	71	22	48	11	
II	52	79	72	57	8	54									
III	14	55													
IV	75	80	59	60	17	37	28	41							
V	5	53	4												
VI	73														
VII	38														
VIII	61	(CDL)													

Table 6. Largest Mahalanobis distances between early lines of group I and II grouped by the Tocher method in the germplasm of common maize. Cascavel, PR, Brazil, 2014-2015.

Lines	Distance range (D2)														
	(400 _ 500)					(500.1 _ 600)					(>600.1)				
	2	7	12	13	18	3	9	31	32	42	6	19	20	40	43
57	23	34	36	39	44	45	46	56	65	68	48	50	58	64	77
	47	51	62	67		70	71	74	78		81	(CDL)			
79	3	31	32	42	43	6	19	20	40	48	58				
	51	56	64	65	68	50	81								
	70	71	74	77											
72	3	19	20	43	50	6	40	48	58						
	56	64	71	74	81										
8	11	31	40	43	48	19	20	58							
	50	77	81												
52	19	20	40	48	50	58									
54	19	40				58									

Genetic correlations between yield components revealed that the magnitudes were low to moderate, and the highest was observed between GW and TGW (r_g = 0.46). There were no high associations between yield components and the variables that indicate earliness. Genetic correlations of GW, TGW, and HW were r_g = 0.07 to 0.24 for AUWLC, r_g = _0.02 to 0.18 for DMF, and r_g = _0.13 to 0.14 for DFF, values classified as very weak and weak. These low magnitudes indicate the possibility of selection for earliness without compromising grain yield (Gasura et al., 2013). The trait EGWR, which is the proportion of grain weight compared to the total weight of the ear, showed variation between 0.58 to 0.91 among the lines evaluated, which were well distributed in the first four groups by the Scott_Knott test (p ≤ 0.05). The lines CDL61 and CDL38 remained isolated from the others, constituting groups "E" and "F". Despite the small number of groups (Table 3), similar to that observed for HW, genetic variability can be used for breeding purposes, since the CVg/CV ratio was 2.10 (Table 1). There were no genetic correlations with high magnitudes for the remaining traits; the highest ones were associated with the yield components. Among the ear attributes (Table 4), there were similarities in groups of the ED and CD traits, which formed eight and nine groups by the Scott-Knott test (p ≤ 0.05), respectively. The ED values ranged from 35.7 to 51.2 mm among the lines studied, and the mean CD values were 21.4 to 32.3 mm. The line considered early for AUWLC, CDL57, was classified in the smaller diameter groups. As observed for flowering, there were similarities of pairs and even groups of lines that remained together for ED and CD (Table 4), associations confirmed by strong genetic correlation between variables (r_g = 0.8).

In regard to yield components, CD had strong genetic correlation with GW (r_g = 0.74). The highest genetic association between the diameters and the variables indicating earliness was between ED and AUWLC, 0.21, of weak magnitude. With respect to CEDR, variation in mean values was from 0.44 to 0.84, distributed into nine groups by the Scott-Knott test (p ≤ 0.05) (Table 4). The ratio indicates the space available in the ear for the grains, and it was investigated for the possibility of promoting water loss, but the trait showed no genotypic correlation of high magnitude with attributes associated with maturity and yield components. The only correlation of high magnitude was with CD (r_g = _0.74), which was negative and expected because of the direct effect of CD in calculating CEDR. For the KRN and EL traits, nine and eight groups were formed, respectively, by the Scott-Knott test (p ≤ 0.05). Lines ranged from 10.4 to 18.7 for KRN, and from 12.5 to 19.9 cm for EL (Table 4). High magnitude associations were found for ear attributes between KRN and ED (r_g = 0.68), KRN and CD (r_g = 0.72), and EL and CEDR (r_g = 0.71), which was expected for these secondary components as they are usually proportional.

The highest genetic correlations the traits showed with attributes related to grain maturity were those between EL and AUWLC (r_g = 0.45), EL and DMF (r_g = 0.52), and EL and DFF (r_g = 0.51). As this association has moderate magnitude, the differences between the results observed and those in the literature are the effect of the genetic variability of each germplasm and the effect of environment.

Multivariate analysis

Through estimation of the relative contribution of traits by the Singh method (1981), the respective importance for genetic diversity was determined in the following descending order: CD (15.4%), EL (14.1%), TGW (10.8%), DFF (10.3%), DMF (9.7%), KRN (9.1%), AUWLC (8.9%), ED (7.8%), HW (4.8%), EGWR (4.7%), GW (3.7%) and CEDR (0.2%). The trait CEDR was of secondary importance given its small contribution; however, its removal caused changes in the composition of groups by the Tocher method, and its permanence in the study of divergence was recommended (Shimoya et al., 2002).

In the Tocher grouping method based on Mahalanobis distances, the lines were assigned to eight groups (Table 5). Group I included 73% of the 81 lines evaluated, followed by groups IV, II, V, and III, which included eight, six, three, and two lines, respectively. Lines CDL73, CDL38, and CDL41 separately constituted groups VI, VII, and VIII, respectively.

Lines CDL08, CDL52, CDL57, and CDL72, previously identified as early for the traits AUWLC, DMF, and DFF by the Scott-Knott test (p ≤ 0.05) were pooled in group II of multivariate analysis by the Tocher grouping method. Lines CDL21, CDL36, CDL46, and CDL64, which stood out for GW in univariate analysis, were also pooled in the same group of multivariate analysis (Table 5).

With the aim of obtaining new early commercial hybrids adapted to the conditions of the second crop season or for the breeding populations (Miranda et al., 2003; Teixeira et al., 2015; Bolson et al., 2016), crossing lines of groups I and II is suggested, provided they belong to distinct heterotic groups (Paterniani, 2001). In order to predict some of these crosses, we considered the longest distances between the lines in the Mahalanobis matrix (Table 6). A few crosses were highlighted with lines CDL8, CDL52, and CDL54. A larger number of crosses were suggested for lines CDL57, CDL72, and CDL79 because of longer distances of several lines between groups I and II. The line CDL57 was the most divergent, with D^2 > 600.0 observed between various genotypes (Table 6).

For cluster analysis by the hierarchical UPGMA, the arbitrary cutoff level was set considering differences (or distances) between the groups. Setting the cutoff to 50% took into account the number of groups obtained by the Tocher method and the best distribution of distances in the dendrogram, projecting the formation of five groups. Group I comprised 71% of the lines, group II, five lines, and group III, thirteen lines. Groups IV and V were formed by single lines, CDL38 and CDL61, respectively (Figure 1).

There was similarity between the groups formed by using the Tocher method and the UPGMA; group I consisted of 55 similar lines. The lines CDL52, CDL57, CDL72, and CDL08 considered early for AUWLC, DMF, and DFF were once more placed in the same group, comprising group III of the UPGMA (Figure 1). In addition, the lines CDL17, CDL60, CDL37, CDL41, and CDL28 of group II of the UPGMA were placed in group IV by the Tocher method. The CDL38 and CDL61 lines formed single groups in both methods. Both clustering methods were considered efficient, revealing genetic variability in the germplasm.

Materials and methods

Material and Cultivation Conditions

The experiment was set up under field conditions at the experimental station of COODETEC (24°53'8.54"S, 53°32'4.72"W, 678 m altitude) in Cascavel, state of Paraná, in the 2014-2015 summer growing season. A total of 81 lines of common maize were distributed according to a square lattice design (9 x 9) with three replications. The experimental unit consisted of four rows of four meters, with between-row spacing of 0.76 m and 20 plants/row.

Features Evaluated

The DMF and DFF was determined at the R1 stage in the entire plot when 50 percent plus one of the plants were releasing pollen and showed exposed style and stigma. Collection of maize for determining the moisture content began at the moment that the earliest lines reached the R6 stage (physiological maturity) and contained around 30% moisture. Three ears were randomly sampled, and moisture content was measured in an automatic moisture content meter, type 999 ES Motomco®. A total of seven samples were taken at four_day intervals over a total of 28 days of recording grain moisture content. Percent values of grain moisture were tabulated in a chart to estimate the AUWLC (Yang et al., 2010). The other traits were TGW, in g; HW, in g; GW, in g; EGWR; ED, in mm; CD, in mm; CEDR; KRN; and EL, in cm.

Statistical analysis

The experimental data were subjected to analysis of variance ($p \leq 0.05$) using the lattice statistical model (Cochran and Cox, 1957). Model efficiency was estimated for all traits, with variations from 95.8% to 104.3%, lower than the 120% limit allowed in running analysis of variance for randomized blocks (Pimentel-Gomes and Garcia, 2002). The genetic and environmental parameters were estimated from this, and means of lines were placed in homogeneous groups, applying the Scott-Knott test ($p \leq 0.05$). Genetic and phenotypic correlations were then estimated to identify the degree of association between them (Cruz et al., 2012).

The behavior of the DMF and DFF variables in response to the AUWLC variable was identified using the linear regression model. Although the linear model was not significant, the means were subjected to the polynomial regression study, which determined which of the polynomials (1st, 2nd, or 3rd degree) satisfactorily explained the relation between flowering and water loss. The F test was used to verify the significance of the polynomial effects, choosing the highest grade model (Pimentel-Gomes, 2009).

Based on the Mahalanobis generalized distance (D^2), we grouped the lines by the Tocher optimization method and the UPGMA and studied the relative importance of traits in relation to genetic divergence between the variables according to Singh (1981). All statistical procedures were performed using the GENES software application (Cruz, 2006).

Conclusion

Water loss determined through the area under the water loss curve provides results with high reliability, but data collection is very labor intensive, which hampers its use in routine procedures of a maize breeding program. The use of female and/or male flowering is not effective for classifying the maturity of maize lines. The assessment of moisture content in a single collection may be an alternative to replace determination of the area under the water loss curve, provided that germplasm has a broad range for grain moisture content at harvest. Low genetic correlations between yield components and maturity attributes indicate the possibility of selection for earliness without compromising yield. Genetic diversity allowed classification of genetic variability in the germplasm and suggests hybrid combinations with higher heterotic effect for earliness and yield.

Acknowledgments

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