STUDY ON COMBINING ABILITY AND USE of SSR MARKER TO DETECT *LG1* AND *LG2* IN ERECT LEAF MAIZE INBRED LINES WITH MO17 AND B73 USING TESTER X LINE MATING DESIGN

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ABSTRACT

The present study was conducted to evaluate the general combining ability effects in a selection of maize inbred lines for grain yield and leaf angle by using tester x line analysis under spring season conditions. Eight erect leaf maize inbred lines and two testers, Mo17 and B73, were crossed in tester x line scheme in the 2014 season. Sixteen testcrosses were evaluated in a randomized complete block design with two replications during the 2015 spring season. Results showed that the E2, E7 and E8 lines had leaf angles from 30^o to 35^o and belong to the compact plant type while the remaining lines had leaf angles (LA) <30^o and belong to the erect leaf plant type. The leaf orientation value (LOV) analysis showed that the plant canopy had vertical leaf orientations in the all lines planted. We identified only one testcross (THL15) that had LA <30^o making it an erect leaf plant type, six testcrosses had LA >35^o making them normal plant types, and the remaining testcrosses belonged to the compact type. Estimates of general combining ability (GCA) effects for the eight inbred lines and the two testers showed that three inbred lines, E4, E7, and E8, and tester Mo17 had small a GCA for leaf angle. There were five inbred lines, E1, E2, E3, E4, and E6, and tester Mo17, that showed a positive GCA for grain yield. The primers *umc1165* (for *lg1*) and *bnlg1505* (for *lg2*) were used to detect the target genes in the parental lines and testcrosses. Results showed that the primers gave PCR products with a high level of polymorphisms so that we could identify that lines and crosses contained *lg1* and *lg2* genes. This suggested that SSR markers could be applied to a MAS program to screen material with erect leaves in order to breed maize for planting in high densities.

Keywords: Combining ability, erect leaf, inbred line

Nghiên cứu khả năng kết hợp và sử dụng chỉ thị phân tử SSR dò tìm gen *lg1* và *lg2* trong lai đỉnh hai dòng thử Mo17 và B73 với các dòng tự phối ngô lá đứng

TÓM TẮT

Nghiên cứu thực hiện đánh giá khả năng kết hợp chung của tám dòng tự phối ngô về tính trạng lá đứng và năng suất hạt sử dụng mô hình line × tester trong vụ xuân 2015; và để phát hiện hai gen *lg1* và *lg2* trong các dòng bố mẹ này cũng như con lai F_1 sử dụng chỉ thị phân tử SSR. Mười sáu tổ hợp lai đỉnh và các dòng bố mẹđược đánh giá trong vụ xuân 2015 trong thí nghiệm khối ngẫu nhiên hai lần lặp lại. Kết quả xác định góc lá trung bình của ba lá trên bắp nhận thấy dòng E1, E5 và cây thử Mo17 có góc lá từ 30-35^o thuộc nhóm lá gọn, các dòng còn lại có góc lá < 30^o thuộc nhóm lá đứng. Giá trị hướng lá (LOV) cũng cho thấy kiểu cây của các dòng và tổ hợp lai thuộc nhóm cây gọn. Chúng tôi xác định chỉ có tổ hợp lai 15 có góc lá <30^o thuộc nhóm lá đứng, sáu tổ hợp lai có góc lá >35^o thuộc nhóm lá thường, và các tổ hợp lai còn lại thuộc nhóm lá gọn. Ước lượng giá trị khả năng kết hợp chung (KNKH) của 8 dòng và 2 cây thử, kết quả cho thấy 3 dòng là E4, E7, E8 và cây thử Mo17 có giá trị âm KNKH về góc lá, nghĩa là góc lá có xu hướng hẹp hơn. Sáu dòng có giá trị KNKH dương về năng suất là E1, E2, E3, E4, E6 và Mo17. Sử dụng chỉ thị SSR với hai mồi đặc hiệu *umc1165* (dò tìm gen *lg1*) và *bnlg1505* (dò tìm gen *lg2*) ở các dòng bố mẹ, THL, kết quả cho thấy mức độ đa hình cao và đã nhận biết được các dòng và THL mang gen *lg1* và *lg2*. Kết quả này gợi ý rằng có thể sử dụng chỉ thị phân tử SSR trong chọn lọc trợ giúp nhờ chỉ thị phân tử (MAS) để sàng lọc vật liệu và chọn giống ngô lá đứng cho trồng mật độ cáo.

Từ khóa: Khả năng kết hợp, lá đứng, dòng tự phối

1. INTRODUCTION

Modern maize hybrid varieties have steadily become more productive throughout the past decades. The increased productivity is partly attributable to higher population densities and genetic adaptations that permit vigorous growth at high planting densities. Because efficient light interception is essential to plant growth, plant growth habits that enable efficient light interception in high population densities increased yield under modern farming conditions (Wassom, 2013). Maize plant architecture is considered to be one of the most important agronomic traits and achieving the ideal plant architecture has long attracted the attention of breeders to improve grain yield. Plant architecture determines planting density and influences photosynthetic efficiency, disease resistance, and lodging resistance.

One of our interests was to investigate the genetic controls underlying leaf angle (LA) by molecular markers for improving maize plant architecture to apply to a MAS maize breeding program. Previous mutant studies have shown that recessive liguleless mutants (*lg1* and *lg2*) and dominant mutations in *knotted1*-like homeobox genes (*Lg3-O, Lg4,* and *Kn1*) are involved in ligule development (Elizabeth M. Buescher *et al.*, 2014). In this study, we evaluated the phenotypic data obtained for LA and leaf orientation value (LOV) using the method described by Ku *et al.* (2010). Li *et al.* (2015) also considered plant architecture to be a key factor for productive maize because ideal plant architecture with erect LA and optimum LOV allows for more efficient light capture during photosynthesis and better wind circulation under dense planting conditions (Li *et al.*, 2015). Researchers from the Crop Research and Development Institute (CRDI) have developed maize inbred lines with erect leaf characteristics. These erect leaf inbred lines were used to evaluate general combining ability using a tester x line mating design with Mo17 and B73. The objective of this research was to select useful lines for breeding hybrid maize with erect leaves adapted to higher planting densities**.**

2. MATERIALS AND METHODS

2.1. Plant materials

Eight newly-developed maize inbred lines from the 4^{th} to 6^{th} selfing generations were selected as parents in this study based on their adaptive traits to high planting density and erect leaves (Table 1). Two lines, CT124 and CT111, were from open-pollinated populations, and six lines, pioneer B3, pioneer B414, pioneer B472, TV175, TV171, and TV169, were commercial single crosses. The two testers were Mo17 and B73 which were obtained from the University of California, Riverside, USA in 2012. B73 was developed by Iowa State University and released 1972 and Mo17 was developed by the University of Missouri and released 1964. Two of the most widely used testers are the Mo17 inbred line from the Lancaster heterotic group and the B73 inbred line from the Reid heterotic group (Uhr and Goodman, 1995).

2.2. Developing the testcrosses

The eight inbred lines and the two testers were planted at CRDI for crossing to create sixteen testcrosses (THL) (Table 2). Self-pollination of each parental inbred was also performed during the same season to obtain enough S_5 to S_6 seeds for further investigation in the next season.

Table 1. Designation, parental source, and origin of the 8 inbred lines (E) and two testers used in this study

Line	Selfing generation	Plant type	Parental source	Origin
E1	S6	Compact	Local variety (CT124)	Vietnam
E2	S6	Erect	Local variety (CT111)	Vietnam
E3	S5	Erect	Commercial variety (pioneer B3)	USA
E4	S5	Erect	Commercial variety (pioneer B414)	USA
E5	S5	Compact	Commercial variety (pioneer B472)	USA
E6	S4	Erect	Commercial variety (TV175)	China
E7	S4	Erect	Commercial variety (TV171)	China
E8	S4	Erect	Commercial variety (TV169)	China
Mo17	Tester	Compact	UC Riverside	USA
B73	Tester	Erect	UC Riverside	USA

Table 2. Parental source and testcrosses in this study

Line♂	Mo17♀	B73♀
E1	THL1	THL9
E2	THL2	THL10
E3	THL3	THL11
E4	THL4	THL12
E5	THL5	THL13
E6	THL6	THL14
E7	THL7	THL15
E8	THL8	THL16

2.3. Evaluation of inbred lines and testcrosses

In the spring season of 2015, field experiments were carried out at CRDI. The experiments were conducted to evaluate twenty four genotypes, namely sixteen testcrosses (THL), eight inbred lines, and two testers (Mo17 and B73). A randomized complete block design with two replications was applied. The experimental plots had 4 rows, each 5 m long with spacing of 0.70 m between rows and 0.25 m within rows. Fertilizer of 160 kg N, 70 kg P_2O_5, and 30 kg K_2O was applied per hectare. Sowing was performed at the beginning of January and harvest was performed in the middle of June.

Data were recorded on (1) days to 50% silking (DTS) (number of days from planting to silking of 50% of plants); (2) anthesis - silking interval (ASI) (number of days between 50% silking and 50% anthesis on 10 plants per plot); (3) plant height (PH), in cm (from ground to the point of flag leaf insertion); and (4) ear height measured on 10 plants from each plot. The yield and yield components were also recorded for lines, testers, crosses, and check variety.

Three leaf traits were collected on plants at maturity. Leaf angle (LA) was measured as the average angle between the blade and stem for the three leaves above the ear. The angle of each leaf was measured from a plane defined by

Gen	Bin	Primer	sequence -forward / reverse
liguleless1(lg1)	2.01	*umc1165*	F: TATCTTCAGACCCAAACATCGTCC/ R: GTCGATTGATTTCCCGATGTTAAA
liguleless2 (lg2)	3.01	*bnlg1505*	F: GAAAGACAAGGCGAAGTTGG/ R: GCTTCTGAACTGGATCGGAG

the stalk below the node subtending the leaf. Maize leaf angles can be classified into 3 groups, according to Kieu Xuan Dam *et al.* (2002), as (1) vertical leaves with leaf angles ≤30°; (2) compact leaves with leaf angles from 30 - 35°; and (3) normal leaves with leaf angles ≥35°. Leaf length (LL) was determined on the three leaves as the length from the beginning of the *ligula* to the tip of the leaf. Leaf orientation value (LOV) was calculated as follows:

$$LOV = \frac{1}{n}\sum(90-\theta)xL_f/LL$$

Where θ is the measured leaf angle, L_f is the length from the beginning of ligula to the flagging point of the measured leaves, LL is the leaf length, and n is the number of leaves measured (Pepper, 1977).

2.4. PCR and gel electrophoresis

This study used SSR markers (Simple Sequence Repeats) to detect gene control of erect leaves. The genes of focus were the *lg1 and lg2* genes with primers according to Ku *et al.* (2011) and James J Wassom (2013). The primer sequences were gained from MaizeGDB as follows:

Total DNA was extracted from young maize leaves of five plants according to Doy & Doy (1990). The young maize leaves were collected from the greenhouse, dried, and ground then ground into a powder. The powder was then placed in 1.5-mL microtubes containing 700 mL 2% CTAB extraction buffer [20 mM EDTA, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl, 2% CTAB], plus 0.4% b-mercaptoethanol added just before use. PCR reactions were as follows: (1) initialization at 95˚C for 5 min; (2) 35 cycles of denaturation at 94˚C for 30 s, annealing at 62°C for 30s, and elongation at 72°C for 2 min; and (3) a final elongation step at 72°C for 5 min. PCR products were separated using gel electrophoresis in a 4% (w/v) agarose gel with 0.5X TAE, stained with ethidium bromide 0.5 µg/ml, observed under UV lamp, and photo-documented with a digital camera

2.5. Statistical analysis

The analysis of variance was carried out using mean values of observations, coefficient of variation (CV), and least significant difference ($LSD_{.05}$) using IRRISTAT ver. 5.0 software. Combining ability analysis using tester × line procedures (Kempthorne, 1957) was performed using the procedure in the quantitative genetic statistical analysis DTSL software (Nguyen Dinh Hien, 1995).

3. RESULTS AND DISCUSSION

In order to evaluate our testcrosses we needed to first analyze a range of agronomical characteristics, including leaf angle, leaf orientation value, grain yield, and yield components, in our eight parental lines and two testers (Table 3). Data recorded in the spring season of 2015 showed that the two testers belong to the early mature group. Sowing to physiological maturity was 101 days in Mo17, and in B73 it was 97 days. In the erect leaf inbred lines, sowing to physiological mature took from 102 to 106 days and thus belong to the medium maturity group. Plant height ranged from 119.1 to 172.7 cm with the tester line B73 being the tallest. Ear height ranged from 32.33 to 51.81 cm and correlated positively with height plant. Our data support labeling three inbred lines as compact based on average leaf angle, E2 (32.68°), E7 (31.86°), and E8 (34.93°), while the remaining lines had leaf angles <30° and belong grouped with vertical leaf types. The leaf orientation value (LOV) ranged from 25.87 (B73 tester) to 38.83 (E5 line) and indicated that the all lines had plant canopies with vertical leaf orientations.

Table 3. Agronomical characteristics of the erect leaf inbred lines and testers grown in the 2015 spring season

Line	GD (d)	PH (cm)	EH (cm)	LA (°)	LOV	ED (cm)	EL (cm)	KRE	KR	KW (g)	GY (t/ha)
Mo17	101	158.3	58.73	21.02	31.31	4.21	14.56	13.6	24.4	228.37	3.03
B73	97	172.7	61.89	19.53	25.87	4.37	15.12	14.3	23.1	201.45	2.94
E1	102	119.7	56.87	17.52	33.93	3.41	13.67	12.7	21.6	172.38	2.53
E2	106	156.6	89.94	32.66	28.68	2.63	13.66	13.2	12.0	229.84	2.42
E3	106	147.3	77.12	21.83	31.17	4.15	14.68	12.3	19.2	165.55	2.91
E4	106	126.8	63.22	23.29	36.95	3.17	13.11	11.2	13.1	170.28	2.69
E5	105	132.3	61.11	23.43	38.83	3.64	15.84	14.5	13.1	171.43	2.95
E6	107	127.7	66.43	27.18	35.11	3.47	15.52	11.6	11.1	200.25	2.15
E7	104	129.3	65.78	31.86	37.25	3.53	13.78	11.7	10.4	182.02	2.76
E8	104	129.3	65.53	34.93	32.15	3.39	14.01	11.9	9.7	176.07	1.48
cv%						*5.12*	*4.24*	*5.75*	*4.35*	*7.00*	*6.17*
$LSD_{.05}$						*0.07*	*0.98*	*0.78*	*0.62*	*9.15*	*0.21*

Note: GD: growth duration (d); PH: plant height (cm); EH: ear height (cm); LA: leaf angle of three top leaves; LOV: leaf orientation value; ED: ear diameter; EL: ear length; KRE: number of kernel rows per ear; KR: number of kernels per row; KW: kernel weight of 1000 grains (g); GY: grain yield per ha (ton.)

Most lines had small ears with diameters ranging from 2.63 to 4.37 cm, grain row per ear ranging from 11.2 to 14.5, and grain number per row ranging from 9.7 grains (E8) to 24.4 (Mo17). Within the erect leaf inbred lines and testers, the ear characteristics included ear lengths ranging from 13.11 cm (E4) to 15.84 cm (E5), ear diameters ranging from 2.76 cm (E2) to 4.52 cm (B73), and 1000 grain kernel weights ranging from 165.55 g (E3) to 228.37 g (Mo17). In general, Mo17 and B73 had ear characteristics higher than those of the erect leaf inbred lines in this study. Differences in the grain yield between the lines and tester were also calculated with grain yield values ranging from 1.48 t/ha (E8) to 3.03 t/ha (Mo17). Results indicated that most agronomical characteristics in the two testers were higher than the erect leaf lines selected at CRDI, and the tester lines performed better that the domestic lines on these characteristics.

Data collected in the 2015 spring season from the crosses is presented in Table 4. Growth duration of THL5 and THL6 were both under 100 days and belong to the early maturity group. The other THLs all had growth durations over 100 days and belong to the medium maturity group. Plant height of the THLs ranged from 185.03 cm (THL11) to 232.50 cm (THL6), and ear height (PH) ranged from 75.66 cm (THL10) to 92.44 cm (THL6) with the proportion of EH to PH about 32% to 46%, which was appropriate. The three THLs that had the longest ear lengths were THL4 (21.11 cm), THL6 (20.18 cm), and THL7 (20.37 cm). The ear diameter ranged from 4.15 cm (THL5) to 5.25 cm (THL9) and the difference was not significant when compared with the two tester lines. Kernel weight of 1000 grains ranged from 238.88 g (THL14) to 288.43 g (THL2), and all the THLs had kernel weights higher than the two testers at a significance level of 5%.

When looking at the leaf characteristics of the THLs, there were four THLs that had leaf angles (LA) and leaf orientation values (LOV) smaller than the parental lines. They were

THL7 (mean LA was 33.17° and LOV was 32.77), THL10 (mean LA was 33.64° and LOV was 35.53), THL14 (mean LA was 33.61° and LOV was 36.12), and THL15 (mean LA was 34.21° and LOV was 34.98). All other testcrosses belonged to compact or normal type plants as the values were higher than the highest values of the parents for LA and LOV. Ear diameters were significantly different between the THLs and the testers. Most of the THLs had higher numbers of kernels per row and higher grain yield than the testers, with the exception of THL7 (21.3 kernel/row; 2.95 t/ha) which was significantly lower than the testers. Two THLs, THL6 (6.49 t/ha) and THL9 (6.30 t/ha), had grain yield higher than 6.0 t/ha.

Based on the LA and LOV results, we divided the testcrosses into groups according to Kieu Xuan Dam *et al.* (2002) as shown in Table 5.

Estimates of GCA effects for the eight erect maize inbred lines and the two testers are presented in Table 6. The results showed that the differences among lines and testers had MS values higher than the Ft at a significant level.

Table 4: Agronomic characteristics of the testcrosses (THL) grown in the 2015 spring season

Testcrosses	GD (d)	PH (cm)	EH (cm)	LA (°)	LOV	EL (cm)	ED (cm)	KRE	KR	KW (g)	GY (t/ha)
THL1	102	220.34	88.14	34.67	39.93	18.76	4.43	14.9	35.4	268.95	5.51
THL2	105	225.38	90.15	34.66	32.79	20.48	4.51	16.3	37.2	288.43	5.86
THL3	100	209.39	83.76	38.61	36.97	18.83	4.70	17.4	34.3	261.09	5.56
THL4	107	213.88	85.55	34.22	30.17	21.11	4.29	17.6	32.2	237.13	5.39
THL5	100	207.59	83.04	39.21	35.75	17.05	4.38	13.7	32.3	267.85	4.57
THL6	98	232.50	93.00	38.41	40.65	20.18	4.52	16.1	37.2	261.86	6.49
THL7	104	219.72	87.89	33.17	32.77	20.37	4.49	14.3	21.3	245.87	2.95
THL8	108	208.21	83.28	34.62	33.50	19.88	4.41	13.6	37.7	248.65	5.62
THL9	105	200.64	80.26	35.48	38.84	17.89	4.99	13.5	36.4	276.25	6.30
THL10	102	203.93	81.57	33.64	35.53	16.81	4.71	14.7	32.9	280.72	5.40
THL11	102	185.03	74.01	37.93	41.07	18.75	4.91	11.6	42.1	286.28	5.80
THL12	101	193.97	77.59	34.52	43.10	17.55	4.63	13.5	36.8	274.49	5.99
THL13	100	187.57	75.03	39.40	38.83	15.86	4.89	12.8	33.7	263.93	4.37
THL14	105	195.82	78.33	33.61	36.12	14.98	4.93	13.5	31.8	238.88	4.95
THL15	106	209.90	83.96	34.21	34.98	15.05	4.70	14.1	25.3	254.11	3.55
THL16	107	207.58	83.03	36.67	38.46	17.75	4.96	14.5	30.0	274.35	4.78
Check	105	211.72	84.69	41.19	39.66	18.76	4.43	15.4	35.4	247.20	6.06
CV%	-	*11.5*	*5.17*	*7.20*	*6.05*	*4.31*	*6.75*	*5.86*	*8.25*	*6.70*	*9.35*
$LSD_{0.05}$	-	*12.75*	*5.21*	*0.56*	*0.80*	*0.87*	*0.55*	*1.55*	*5.12*	*15.23*	*0.33*

Note: GD: growth duration (d); PH: plant height (cm); EH: ear height (cm); LA: leaf angle of three top leaves; LOV: leaf orientation value; EL: ear length (cm); ED: ear diameter (cm); KRE: number of kernel rows per ear; KR: number of kernels per row; KW: kernel weight of 1000 grains (g); GY: grain yield per ha (ton.)

Table 5. The leaf architecture of the testcrosses between Mo17 and B73 with erect leaf inbred lines

Line	E1	E2	E3	E4	E5	E6	E7	E8
Tester	(1)	(2)	(1)	(1)	(2)	(1)	(2)	(2)
Mo17 (1)	3	2	2	2	3	3	2	2
B73 (1)	2	2	3	2	3	3	1	2

Note: (1) leaf angles ≤ 30 º; (2) Compact leaves with leaf angles from 30 - 35º, and (3) normal leaves with leaf angles ≥ 35º.

Table 6. Analysis of variance for leaf angle

Source of variance (S.O.V)	df	SS	MS	Ft
Block	1	0.289	0.289	1.061
Testcrosses	15	27.762	1.851	6.800
GCA line	7	14.100	2.014**	1.507
GCA tester	1	4.307	4.307**	3.223
SCA tester x line	7	9.355	1.336	4.910
Error	15	4.083	0.272	
Total	31	32.133		

Table 7. Analysis of variance for grain yield and and their combined data

Source of variance (S.O.V)	df	SS	MS	F
Block	1	0.289	0.289	0.016
Crosses	15	27.025	1.802	75.785
GCA line	7	22.873	3.268*	5.605
GCA tester	1	0.070	0.070*	0.121
SCA tester x line	7	4.081	0.583	24.524
Error	25	0.594	0.024	
Total	51	32.456		

Contribution rate of the lines and testers to the general variance showed that lines contributed 50.788%, testers contributed 15.514%, and testers x lines contributed 33.697%. Difference in the GCA value of the tester Mo17 is -0.367 and B73 is 0.367 at a significant level (error is 0.130). The proportional contribution of lines, testers, and their interaction to the total variance showed that lines played an important role in the total variance for all traits, indicating a predominant line influence.

Contribution rate of the lines and testers into general variance for grain yield showed that lines contributed 84.639%, testers contributed 0.260% and testers x lines contributed 15.101%. Based on the overall performance of the hybrids and parental lines, some of the lines could be used as parents of hybrids of maize with erect leaves and moderate yield potential.

Estimates of GCA effects for the eight erect maize inbred lines and the two testers are presented in Table 8. Results showed that three inbred lines, E4, E7, and E8, and tester Mo17 possessed negative (desirable) and significant GCA effects for leaf angle toward narrowness. Six inbred lines, E1, E2, E3, E4, E6, and Mo17, showed a positive GCA for grain yield (demonstration in Table 8 and Figure 1). The lines that possessed negative (desirable) and significant GCA effects for height plant toward shortness were E6 and E7, while E1 had a positive GCA and significant GCA effect for plant height. All other lines had non-significant GCA values for this trait. E5, E7, E8, and Mo17 tester line possessed negative (desirable) and significant GCA effects for ear height toward shortness.

Table 8. General combining ability of the erect leaf inbred lines and testers grown in 2015 spring season

Line	General combining ability (GCA)			
	leaf angle	Grain yield	Plant height	Ear height
E1	0.492*	0.718*	15.269*	1.021*
E2	0.282^{ns}	0.476*	4.044^{ns}	1.818*
E3	0.337*	0.498*	12.454^{ns}	1.268*
E4	-0.908^{ns}	0.463*	6.374^{ns}	1.568*
E5	0.657*	-0.742^{ns}	-5.306^{ns}	-0.732*
E6	0.702*	0.528*	-18.090*	-0.032
E7	-0.533^{ns}	-1.919^{ns}	-15.896*	-2.832*
E8	-1.028^{ns}	-0.022^{ns}	1.149^{ns}	-2.082*
Mo17	-0.367^{ns}	0.047*	-2.568^{ns}	*-0.469**
B73	0.367*	-0.047^{ns}	2.568^{ns}	*0.469**
CV (%)	*0.69*	*0.055*	*17.612*	*0.470*
$LSD_{0,05}$	*0.261*	*0.039*	*12.453*	*0.332*

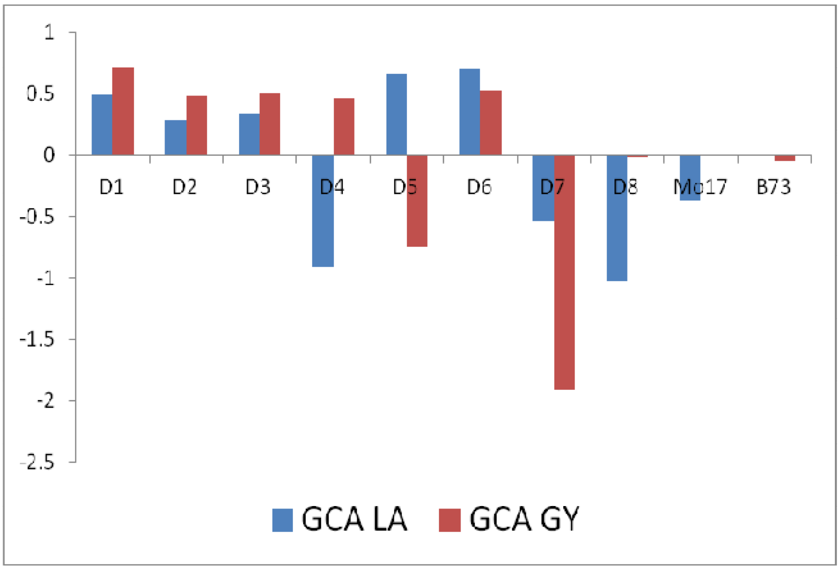

Figure 1. GCA effects for leaf angle and grain yield of parental lines and testers

This study used SSR markers with specific primers according to those previously reported by Wassom (2013) to detect gene control of leaf angle in the strong candidate genes *lg1* (*liguleless-1*) and *lg2* (*liguless-2*). The *lg1* mutant has no ligule or auricle, leading to considerably more upright leaves than their normal counterparts. The mutant phenotype and expression analysis of *lg2* suggest an early role in initiating an exact blade-sheath boundary within the young leaf primordial (Walsh *et al.*, 1998). Results showed that primer *umc1165* (for *lg1*) identified an allele approximately 650 bp in size and primer *bnlg1505* (for *lg2*) identified two alleles ranging in size from 150 to 200 bp showing that this marker gained a polymorphism . These results confirm that the parental lines contain the *lg1* and *lg2* genes.

Detection of the *lg1* and *lg2* genes on the 16 THLs was also conducted with two primers as above for leaf angle. Results showed smaller polymorphisms than parental lines and identified three alleles in 15 of the THLs (THL5 did not have a band). Alleles were about 600 - 700 bp in size (Figure 3).

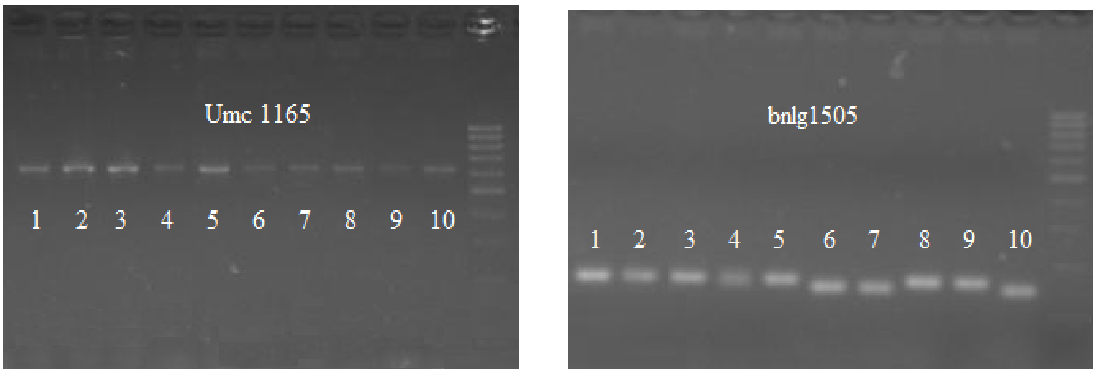

Figure 2. DNA band pattern amplified by the two marker primers *umc1165* and *bnlg1505* of the eight erect leaf lines and two testers

Note: M is the 100 pb Promega DNA ladder which indicates the polymorphic band of 150 bp

Well	1	2	3	4	5	6	7	8	9	10
Line	Mo17	B73	E1	E2	E3	E4	E5	E6	E7	E8

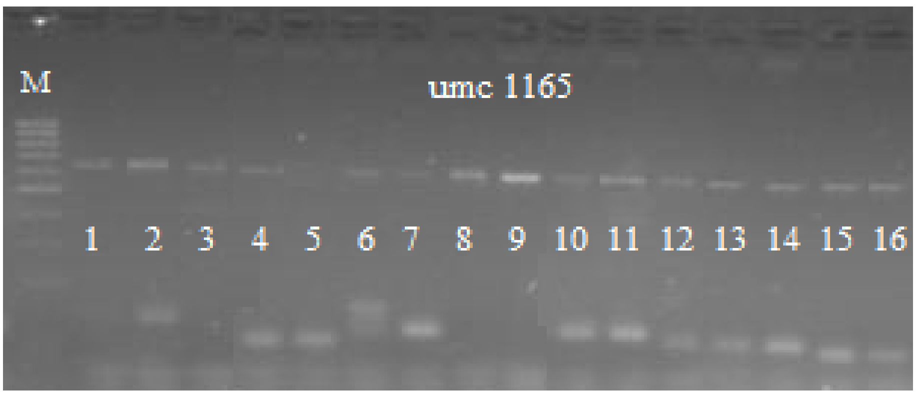

Figure 3. DNA band pattern amplified by the two primers for marker *umc1165* in the 16 crosses

Note: M is the 100 pb Promega DNA ladder which indicates the polymorphic band of 350 bp

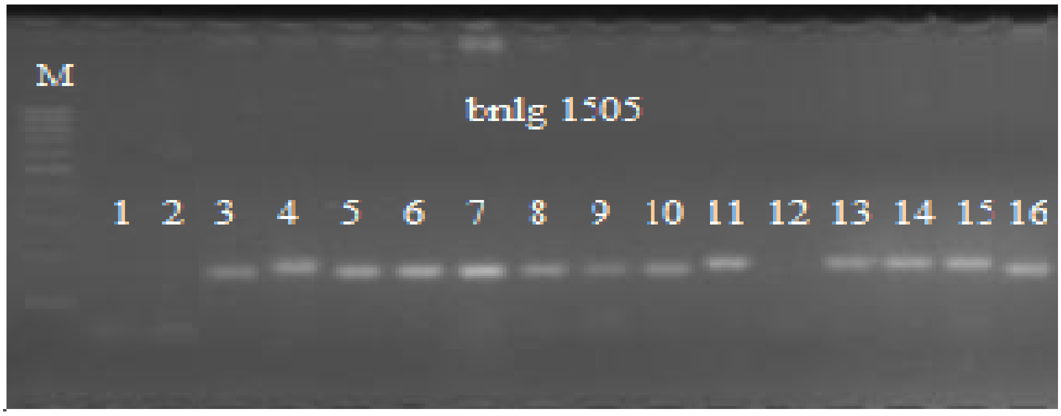

Figure 4. DNA band pattern amplified by the two primers for marker *bnlg 1505* in the 16 crosses

Note: M is the 100 pb Promega DNA ladder which indicates the polymorphic band of 350 bp

Well	1	2	3	4	5	6	7	8
Crosses	THL1	THL2	THL3	THL4	THL5	THL6	THL7	THL8
Well	9	10	11	12	13	14	15	16
Crosses	THL9	THL10	THL11	THL21	THL13	THL14	THL15	THL16

Primer *bnlg1505* detected *lg2* and identified three alleles within the 13 THL. Three THLs, THL1, THL2, and THL12, did not have an observable band. Alleles were about 180 - 200 bp in size (Figure 4). Our results suggested that the SSR marker for *lg2* could be used for MAS in material screening for erect leaves in a maize breeding program looking at high density planting. The information from this study may be useful for researchers who would like to develop high yielding and high erect leaved maize inbred lines and hybrids.

4. CONCLUSION

Results showed that the two testers belong to the early maturing group and the erect leaf inbred lines, with growth durations from 102 to 106 days, belong to the medium maturing group. Leaf angle measurements identified three compact inbred parent lines, E2, E7 and E8, while the remaining lines had leaf angles <30° and could be classified as having vertical leaves. The leaf orientation value (LOV) indicated that all the lines tested had plant canopies with vertical leaf orientations.

The testcrosses belonged to the medium maturity group based on their growth durations. There were four testcrosses that had leaf angles and leaf orientation values smaller than the parental lines while the other testcrosses belonged to the compact or normal canopy type.

Estimates of GCA effects for the eight erect maize inbred lines and the two testers showed that three inbred lines, E4, E7, and E8, and tester Mo17 possessed negative (desirable) and significant GCA effects for leaf angle toward narrowness. There were six inbred lines, E1, E2, E3, E4, E6 and Mo17, that showed a positive GCA for grain yield,.

The primers *umc1165* and *bnlg*1505 were used to detect the lg1 and lg2 genes, respectively, on the parental lines and crosses grown in the spring of 2015. Results showed that the primers gained polymorphisms so we were able to confirm that the parental lines, crosses, and check variety contained the *lg1* and *lg2* genes. This suggests that the SSR markers are useable to identify the erect leaf phenotype in maize and can be used in a maize breeding program for high density planting.

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