International Journal of Agricultural Sciences ISSN 2167-0447 Vol. 10 (7), pp. 001-006, July, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Combining ability studies amongst AREX and CIMMYT maize (*Zea mays L.*) inbred lines under stress and non stress conditions

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Accepted 22 November, 2019

Maize (*Zea mays* L.) is the staple food crop in Zimbabwe with a per capita consumption of 93 kg. It is grown in a wide range of environments with 80% being produced by the smallholder farmers who occupying more than 90% of the marginal areas of the country. Marginal area production has seen a high hybrid turnover on the market hence the need to develop hybrids with stable yields under diverse environments. National program use of inbred lines as testers has had shortcomings in the early identification of good inbred lines, resulting in slow variety development, poor commercial seed production and eventual variety release for farmer use. The objectives of this study were to develop single cross hybrid testers among AREX and CIMMYT lines as well as determine the heterotic relationship among the two sets of inbred lines. In the study, testcross development was done using 10 elite inbred lines each from AREX and CIMMYT programs. Using North Carolina Design II the resultant 100 hybrids were evaluated under optimum and stress (low N and drought) environments. Alpha (0,1) lattice design was used in the evaluation process with traits such as flowering dates, standability, disease scores, plant heights and grain weight being recorded. An across site analysis was done and results showed that there were significant differences (P<0.05) for environments, genotypes and genotype x environment interactions. Significant general combining ability (GCA) effects for all the traits (P<0.05) measured except for plant heights and stem lodging were observed, with five lines being identified as having good (positive) GCA effects for grain yield. Non additive genes were also predominant in most traits except for anthesis dates, anthesis silking interval and ear heights. A total of 39 testcrosses were also assigned heterotic groups basing on the N and SC heterotic groups. Tester identification was based on good GCA for grain yield, stability under diverse environments and maturity of genotype. In the N heterotic group, genotype LT52 (NAW5885/CMML442) was identified as a potential single cross tester in the intermediate maturity group while in the SC heterotic group genotype LT26 (SC5522/ZM621A-BBBB) was identified as another intermediate maturity group tester. In the early maturing category the only possible candidate identified was LT99 (RS61P/CML508) which is in the SC heterotic group. The study also showed that there were heterotic group overlaps of the N and SC groups in relation to CIMMYT's A and B heterotic groups as some genotype combinations had to be assigned new heterotic groups or had their group unidentified resulting in the need for further evaluation.

Key words: *Zea mays*, inbred lines, combining ability, heterotic groups, AREX, CIMMYT.

INTRODUCTION

In Zimbabwe, maize production accounts for 80% of the total cereal crop. The crop is widely grown in varying environments with a total of 1.2 million hectares having been put to maize during the 2004/05 season (AREX, 2004). Normal annual production ranges from 1.8 to 2.1 million tonnes with a yield average of 1.2 tha^{-1} and 4.5 tha^{-1} in the smallholder and large scale commercial sectors respectively. According to CIMMYT (1990)

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improved yields, variety yield stability, pest and disease resistance, tolerance to drought and low soil fertility, generally produce yield improvements of 30-50%. There is therefore a need to develop stress tolerant varieties especially for the smallholder, stress prone environments. The national maize breeding program in Zimbabwe has been trying to solve some of the production constraints highlighted, through the development of maize varieties with drought, low N and disease tolerance.

However the national program hybrid development pace has been slower than the hybrid turnover. The hybrid turnover especially in the 1990s had been very high due to frequent droughts and diseases such as gray leaf spot *(Cercospora zea maydis)* and maize streak virus. The slow variety release progress has been compounded by the use of inbred line testers. The Zimbabwean maize hybrid market is mainly three way hybrids. Development of such varieties is longer when using inbred line testers as there is need for a third parent after the single cross development thereby delaying improved hybrid access by farmers. The two testers currently in use are known to be susceptible to the gray leaf spot and streak virus diseases. The two inbred line N3.2.3.3 and SC5522 testers cum heterotic groups were developed from open pollinated varieties namely Salisbury White and Southern (Olver, 1988; Doswell et al., 1996). These inbred line testers are however late maturing and hence do not fit very well in the early maturing breeding programs. Single cross testers are ideal in the early identification of good inbred lines in a breeding program as well as in commercial maize seed production.

Development of single cross testers through use of elite CIMMYT inbred lines which are known to be biotic (maize streak virus and gray leaf spot) and abiotic (drought and low N) stress tolerant would widen the national germplasm pool for biotic stress resistance and abiotic stress tolerance selection. Introgression of exotic germplasm is often suggested as an approach to increase genetic differences between opposing heterotic populations, thereby potentially increasing heterotic response (Mickelson et al., 2001). Therefore, the objective was to develop single cross testers for a hastened variety development. These single crosses would also aid in the early identification of potential good inbred lines as well as develop market oriented three way hybrid in a shortest possible time. The use of elite national and CIMMYT inbred lines also help in determining the heterotic relationships of the two breeding programs.

MATERIALS AND METHODS

Ten elite inbred lines from National Breeding Program, AREX and ten from the Maize and Wheat Improvement Centre CIMMYT were crossed in Winter 2005 (Table 1). The North Carolina Design II method was used in the crossing program to give a total of 100 crosses, with reciprocal crosses being bulked. This method is employed where there is a fairly large size of germplasm and where there are established heterotic groups proven testers. According to Gutiereez-Gaitam et al. (1996), this technique is used to broaden the genetic basis of established heterotic groups with germplasm of similar heterotic response. Inbred lines N3.2.3.3 and SC5522 (national) and CML312 and CML395 (CIMMYT) were used in the design as standards for the heterotic group classifications. Below is the table that shows the parental lines used in the study.

Environments

The experiment was evaluated in two non-stress (optimum) and two stress (drought and low N) sites in Zimbabwe (Table 2). In the optimum environments, fertilizer, herbicides and insecticides were applied according to practices that would provide optimum growing conditions at each site.

Trial management

The two optimum trials and the low N trial were planted during the summer 2005/06 season (Nov 05 – April 06) with the drought trial evaluation being done in winter 2006 (May- October 2006). Low N block soil analysis results showed 4 ppm N in the top 30 cm which translates to approximately 30 kg N per hectare which is about 25% of the required N under optimum conditions. Available P_2O_5 was 57 ppm which is ideal (>50 ppm) for optimum plant growth hence no P was added. Exchangeable cations me/100 g were 0.24 for potassium (K), 8.74 for calcium (Ca) and 4.99 for magnesium (Mg). All were above the threshold for optimum plant growth in a reddish brown clay soil but a maintenance dressing of 20 kg/ha KSO_4. At Save Valley site, drought was managed through irrigation at critical times only. A total of 280 mm irrigation was applied in the first 8 weeks of crop's growth. This resulted in drought coinciding with flowering and grain filling.

Experimental design

The testcross evaluation was done using the Alpha (0, 1) lattice design. Each of the 100 single cross hybrids was planted in one row plot; 4 m long, while a spacing of 90 cm between rows x 30 cm between plants within rows was used in all the four environments. Trials were replicated three times, with each entry having two seeds per station planted and later thinned to give a plant population of 48 000 plants ha^{-1}.

Data were recorded for grain yield (GY) (Mg ha^{-1}) with grain moisture adjustment of 12.5% at harvest, days to anthesis (AD) (number of days after planting to when 50% of plants start shedding pollen or had extruded silks), plant height (PH) (centimeters from base of stem to insertion of first tassel branch), percent lodging (RL/SL) (calculated as number of plants visibly root/stalk lodged at harvest), anthesis silking interval (ASI) (anthesis date and silking date differences per entry), ears per plant (EPP) (calculated as a ratio of the number of ears with at least one full kernel divided by the number of plants harvested per entry).

Data analyses

Individual site analysis of variance (ANOVA) was done before a combined analysis using SAS (SAS Institute, 2001) and this enabled the performance of the crosses to be assessed under stress and non-stress conditions. The main criterion used for the choice and grouping of the materials was the performance of the testcrosses made between the known heterotic groups. The performance measurements of the testcrosses were based on the

Table 1. Inbred lines and their respective heterotic groups.

AREX		CIMMYT	
Line	**Heterotic group**	**Tester**	**Heterotic group**
N3	N	CML395	B
Sc	SC	CML442	A
2Kba	SC	CML444	B
K64r	SC	CML202	B
NAW5885	N	CML445	B
SV1P	SC	ZM621A-BB	A
WCOBY1P	SC	CML505	A
2N3d	N	CML504	A
RS61P	SC	CML508	A
RA214P	N	CML509	A

Table 2. Site characteristics during the evaluation seasons.

Site	Location	Average T* (°C)	Total rainfall (mm)	Soil type
Harare	17.48°S 31.04°E 1506 m asl	25.4	880	Rhodustalf Group 35 - 55% clay
Gwebi	17.13°S 31°E 1406 m asl	24.8	920	Rhodustalf Group 35 - 55% clay
Save Valley	20°S 33°E 455 m asl	29.7	425**	Sandy Soil

*Average temperature during evaluation ** Preceeding 2005/06 summer rainfall received.

Table 3. Across site analysis mean squares for GY and agronomic traits with GCA predominance.

Source of variation	Df	GY	AD	ASI	EH
Environment	3	2886.44***	14719.69***	54.88***	163718.7***
Genotype	99	4.18***	89.68***	14.25***	1142.99***
Gen x Env	297	2.97**	20.9***	6.04***	403.82**
GCA	18	6.97***	611.22***	101.25**	6170.47**
SCA	81	1.64***	17.66***	4.53*	1.34*
Error	799	2.28	7.35	3.37	213.79

Indicates significance at *P=0.05: **P=0.01; *** P=0.001 and ns: not significant. KEY: GY = Grain yield, AD = Anthesis Day, ASI = Anthesis Silking Interval, EH = Ear Height.

values of General Combining Ability (GCA) and Specific combining Ability (SCA) effects. Heritability of traits were calculated using the narrow sense heritability estimate for estimating genetic variances with assigning of inbred lines to heterotic groups, being done using SCA effects where positive SCA effects between inbred lines generally indicates that inbred lines are in opposite heterotic groups while inbred lines in the same heterotic group exhibit negative SCA effects (Vasal et al., 1992).

RESULTS AND DISCUSSION

Significant differences (P<0.05) for grain yield and other agronomic traits were detected for environment and genotype. Genotype x environment interactions were significant (P<0.05) for all the traits measured except plant height, root lodging, ears per plant and ear rots (Table 3). Environmental differentials indicated that all the four environments were unique. In this study three traits namely anthesis date anthesis –siking interval and ear height had a predominance of GCA sum of squares to SCA sum of squares with the traits anthesis date, anthesis –silking interval, ear height and plant height having a predominance of GCA variance to SCA variance. Traits with additive gene action where GCA sums of squares and variances were predominant over SCA sums of squares and variances are shown in Table

Table 4. Across site analysis mean squares other agronomic traits with SCA predominance.

Source of variation	Df	PH	RL	SL	EPO	EPP	ER
Environment	3	557497.62***	25.35^{ns}	998.73***	1.22***	21.36***	2576.13***
Genotype	99	5623.32***	13.96^{ns}	184.22***	0.01***	0.06**	67.86**
Gen*Env	297	3811.35^{ns}	12.48^{ns}	93.33*	1.37**	0.04^{ns}	54.59^{ns}
GCA	18	17527.87***	37.83**	624.41***	0.04***	0.14***	98.27*
SCA	81	3820.74^{ns}	11.72^{ns}	129.51***	0.004^{ns}	0.05**	53.56^{ns}
Error	799	3452.07	13.07	82.29	0.003	0.04	45.69

Indicates significance at *p = 0.05: **p = 0.01; *** p = 0.001 and ns: not significant.

Table 5. GCA affects values for GY and other agronomic traits measured.

Line	GY (t/ha)	AD (Days)	ASI (days)	PH (cm)	RL (%)	SL (%)	EH (cm)	EPO (cm)	EPP (0-1)	ER (1-5)
1	0.08	1.67	0.34	5.15	0.35	0.51	2.22	-0.003	-0.03	-0.29
2	0.29	2.79	1.51	18.19	0.24	0.69	13.62	0.023	-0.01	0.69
3	-0.22	-0.23	0.29	-5.14	-0.60	0.77	-1.29	0.003	-0.01	1.49
4	-0.37	-0.24	-0.87	-8.06	0.19	-0.23	-3.60	-0.002	-0.00	0.36
5	0.36	0.31	0.56	3.84	0.02	-1.81	1.58	-0.003	-0.02	0.23
6	-0.33	-4.89	-1.64	-18.22	1.26	5.32	-13.03	-0.030	-0.00	1.29
7	0.02	-1.59	-0.47	-11.04	-0.45	-2.17	-4.27	-0.003	0.04	-1.06
8	-0.01	2.90	-0.27	5.77	-0.03	1.09	8.03	0.025	-0.05	0.08
9	0.15	-0.85	-0.43	-8.12	-0.36	-1.66	-0.61	0.013	0.07	-1.74
10	0.06	0.08	0.96	17.41	-0.60	-2.38	-2.70	-0.023	-0.001	-1.02
LSD (0.05)	**0.034**	**0.108**	**0.05**	**50.821**	**0.192**	**10.903**	**3.147**	**0.0004**	**0.001**	**0.673**

3 while those with SCA variances predominant over GCA variances (non additive gene action) are shown in Table 4. According to Melchinger (1998), if the predominance of GCA sum of squares to SCA sum of squares translates to a ratio where GCA variance predominates SCA variance, then early testing of genotypes becomes more effective and promising hybrids can be selected based on their prediction from GCA effects. It therefore implies that early testing of lines selected from the testcrosses from the study pool can be done for traits anthesis date, anthesis silking interval and ear height because of their predominance of GCA variances to SCA variances. Early testing of the lines is more effective because additive gene action is not affected by inbreeding depression. Inbred lines that are under control of additive gene action will therefore not suffer from inbreeding.

On the SCA effects, Vasal et al. (1992) stated that lines in the same heterotic group exhibit negative SCA effects when crossed to each other while those in different heterotic groups show positive SCA effects. Based on the above criterion and using N and SC heterotic groups, across site SCA effects for GY were used to determine to which heterotic group each test crosses could be assigned. A total of 22 and 34 testcrosses were assigned to the N and SC heterotic groups respectively.

Table 5 shows that across locations GCA effects also significant for all traits measured except for plant height and stalk lodging. Lines L1, L2, L5, L7, L9 and L10 were found to have the desired GCA effects. This implies that these lines had good general combining ability with the different tester used hence their progenies had good performance across environments. L1, L5 and L10 are in the N heterotic group, while L2, L7 and L9 are in the SC heterotic group.

In this study narrow sense heritability is reported and the estimates were as shown in Table 6. The estimates for GY, AD, ear position (EPO) and EPP compare very well with those reported by Hallauer and Miranda (1981) where they recorded 18.7% for GY, 57.9% for AD, 39% for EPP and 66.2% for EPO.

Looking at the Abiotic stress related traits of AD and ASI have high heritability hence these traits are readily transmitted and direct phenotypic selection can be done since there are more additive gene than non additive gene effects governing these traits. EPP another abiotic stress related trait, has low heritability implying direct phenotypic selection for this trait is not promising. Use of phenotypes from relatives and selection indices can be employed to determine more accurately its underlying genetic merit (www.cau.edu.cn).

Table 6. Across site: trait means, GCA and SCA variance and heritability.

Trait	Across means	GCA variance	SCA variance	Heritability (%)
Grain yield (Mgha^{-1})	4.86 ± 0.2	1.30	7.52	21.20
Anthesis dates (d)	72.7 ± 0.1	109.28	55.04	55.40
Stem lodging (%)	5.63 ± 0.6	78.48	251.84	33.40
Ear height (cm)	92.9 ± 1.6	1252.32	386.24	70.10
Anthesis silking interval (d)	2.98 ± 0.0	13.92	6.24	71.00
Plant height (cm)	207.2±25.9	2685.76	1966.24	31.60
Root lodging (%)	0.81 ± 0.01	3.04	7.20	1.20
Ear position (0-1)	0.45 ± 0.00	0.01	0.01	70.60
Ears personal plant	0.84 ± 0.00	0.02	0.05	16.90
Ear rots (%)	13.7 ± 0.3	15.92	41.92	15.20

GCA effects

Significant GCA effects (P<0.05) were observed for GY which suggest the need of selecting the genotypes from lines with the best positive effects for consideration as testers. Lines L1, L2, L5, L7, L9 and L10 had positive GCA effects for GY, which implies that the lines contributed to an increase in yield for the testcrosses which was above the mean of the trial. In addition these desirable lines had significant GCA effects among themselves with the exception of L1 and L10 which had similar GCA effects for GY. L3, L4 and L6 had negative GCA effects for yield because they were early maturing as evidenced by their negative GCA effects for AD. Early maturing germplasm has been reported to yield less in general, due to reduced photosynthetic and assimilate accumulation period. Lines L7 and L9 also had negative GCA effects for AD but positive GCA for GY because they had a longer grain filling period since they are intermediate in maturity hence an above trial mean GCA effect for GY. Half the number of lines evaluated had negative GCA effects for ASI which is a desirable feature in the resultant genotypes. Negative ASI GCA effects mean that the lines conferred better synchronization to their genotypes. Despite having a desirable GCA effects for ASI, L4 and L6 yielded low, due to GY penalty that comes with earliness. Intermediate maturing L7 and L9 had negative GCA effects for ASI indicating good nicking properties hence the positive GCA effects for GY. On the contrary L8 had negative GCA effects for ASI but is late maturing hence the below trial mean GY performance associated with late maturity especially under stress environments as evidenced in studies by Edmeades et al. (1998).

Considering the line GCA effects for GY, in the N heterotic group the lines L5, L1 and L10 had positive GCA effects with the best GCA effects being for L5 which conferred a yield of 0.36 Mgha^{-1} above the across site mean yield. Genotype LT52 which is intermediate in maturity (66 days) under optimum environments was identified as a potential tester because it has been noted to have good GCA effects for GY and stability in GY under diverse environments. L1 with a GCA effect of 0.07 Mgha^{-1} is the second best general combiner in the N group series.

Two genotypes LT11 and LT13 can be selected to be good bets for testers. However their average yields under optimum conditions were below the trial mean of 9.24 Mgha^{-1}. The GCA effects for L10 were 0.06 Mgha^{-1} above the grand mean, with genotype LT103 being the only possible tester candidate.

L2, L7 and L9 were the lines with the best GCA effects in the SC group. Genotypes developed from L2 had the best GCA effects of 0.29 Mgha^{-1} above the across site trial mean. Genotype LT26 was the best across all environments, with LT25 being the best bet as tester under stress environments. Genotypes from L9 had a GCA effect of 0.15 Mgha^{-1} and LT95 is the best bet as a tester. It is a good yielder under optimum conditions under which seed production is done as well as a good performer under stress environments. LT99 can also be considered for its stress tolerance and early maturity with L72 and LT79 being other tester candidates in the L7 series of genotypes.

After consideration of GCA effects for GY and other traits like AD, ASI and EPP, and SCA effects for GY under optimum and stress environments, five genotypes shown in Table 7 were considered to be the best bets as single cross testers as they showed good GCA for GY, AD, ASI and EPP traits, good performance across environments and earliness in maturity ideal for the national breeding program.

Table 7. Testcrosses identified as candidates for testers.

Group			Line	Tester	SCA effects				
Genotype	Heterotic	Maturity				Low N	Drought	Across	Optimum yield
LT52	N	Intermediate	0.36	0.11	1.45	3.66	2.91	-0.62	10.35
LT103	N	Intermediate	0.06	0.54	1.52	3.13	3.21	-0.01	11.24
LT26	SC	Intermediate	0.29	0.15	0.79	0.6	3.45	-0.33	10.97
LT95	SC	Intermediate	0.15	0.02	0.54	0	0.48	-0.15	9.92
LT99	SC	Early	0.15	-0.51	1.02	0.31	0.23	-0.34	7.87

Conclusion

Heritability estimates given in the discussion are specific to the germplasm and environments under study. The North Carolina Design II was effective and ideal in the identification of lines with good GCA effects which consequently enabled the identification of potential single cross testers. In the intermediate category LT52 (N group) and LT26 (SC group) can be used as tester while in the early maturing range only genotype LT99 was identified as possible single cross tester. In the study, non additive genes are predominant in most traits with additive gene effects being predominant in AD, ASI and EH only. The study however did not manage to confirm the distinctness between N and SC heterotic groups in relation to CIMMYT's A and B heterotic groups as some combinations had to be reassigned new heterotic groups. As a result, further investigations on inbreds and single crosses that showed positive GCA is needed to confirm their performance and confirm heterotic groupings. Traits used to assess stress tolerances especially ASI and EPP were also used in aiding the identification of potential single cross testers. Further trials to confirm the identified single cross testers need to be done. This is also supported with studies by Vasal et al. (1992) which also stressed the need to do more than one evaluation to identify good lines and testers in tropical maize germplasm. There is need to do further evaluations to establish heterotic groups of genotypes that were not classified into heterotic groups. Off shoots of the study which include hybrids with good SCA such as LT27 can be used by the national program as possible candidates for release or for use in the development of three way hybrids that can there after be released to farmers for commercial production.

ACKNOWLEDGEMENTS

Rockefeller Foundation for financially supporting this research work. Special thanks also go to the CIMMYT Zimbabwe, Ministry of Agriculture (AREX) Zimbabwe and UNZA Crop Science Department

REFERENCES

AREX (2004). Crop Assessment and Early Warning Unit Seasonal Report, Harare, Zimbabwe p. 25.

CIMMYT (1990). The CIMMYT Maize program Information Packet. Mexico. D.F.

Doswell CR, Paliwal RL, Cantrell RP (1996). Maize in the Third World. Westview Press. U.S.A.

Edmeades GO, Bolanos J, Banziger M, Ribaut JM, White JW, Reynolds MP, Lafitte HR (1998). Improving crop yields under water deficits in the tropics. In: Chopra, V.L., Singh, R.B and Varma, A (Eds) Crop productivity and sustainability-shaping the future. ICSC/IBH, Oxford, UK/New Delhi, India. pp. 437-451.

Gutirrez –Gaitam MA, Corttez- Mendoza H, Wathika EN, Gardner and Darrah LL (1996). Testcross evaluation of Mexican maize populations. *Crop Science*, 26: 99-104.

Hallauer AR, Miranda JB (1981). Quantitative genetics in maize breeding. Iowa State University Press. Ames. Iowa.

Mickleson HR, Cordova H, Pixley KV, Bjarnason MS (2001). Heterotic Relationships among nine temperate and subtropical maize populations: Crops Science, 41: 1012-1020.

Olver RC (1988). Maize breeding in Zimbabwe: In: H.O. Gevers. 1998. Proceedings of Eleventh South African Maize Breeding Symposium, pp. 22-27.

SAS Institute (2001). SAS system for windows version 8.2. SAS Institute. Cary, NC.

Vasal SK, Srinivasan G, Pandey S, Beck DL, Crossa J, De Leon C (1992). Heterosis and combining ability of CIMMYT's tropical late white maize germplasm. *Maydica* 37: 217-223.