Combining ability amongst CIMMYT's early maturing maize (*Zea mays* L.) germplasm under stress and non-stress conditions and identification of testers

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Abstract CIMMYT (International Maize and Wheat Research Institute) Zimbabwe's early maturing maize program, which aims to supply seed to approximately 4 million hectares of maize area in eastern and southern Africa, lacks adequate information on heterotic relationships among early maturing germplasm and has no early maturing testers for hybrid development. Open-pollinated varieties (OPVs) and hybrids are the products targeted for this region. Among the hybrids, three-way and double-cross hybrids are desired. Thus the use of single crosses as testers would be an appropriate choice for such a breeding program as one could potentially identify three-way combinations during the early generation test. A twelve-parent diallel was formed and crosses evaluated to identify heterotic groups and single-cross testers. Crosses were evaluated under four different environments in Zimbabwe, two optimal, one low nitrogen stress and one drought stress. P5 (an early maturing line from heterotic group A) and CML 395 (a late maturing inbred line from heterotic group B) were used as reference parents to establish heterotic groupings of germplasm used in the diallel. The single cross (P7/P8) was identified as a potential group A tester because of: (a) co-classification of inbred lines into heterotic group A, (b) good yields- 9.8 t/ha (optimal), 3.4 t/ha (low nitrogen) and 2.1 t/ha (drought); and (c) good GCA effects for grain yield (0.49 t/ha) of line P7 while line P8 contributed to reduced height and anthesis-silking interval.

Keywords General combining ability · Specific combining ability · Testers · Heterotic groups · Combining ability effects

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Introduction

Maize, an important food crop in Africa, especially in sub-Saharan Africa, provides 50% of the calories in diets in southern Africa, 30% in eastern Africa and 15% in West and Central Africa (Zambezi and Mwambula 1997). Frequent maize shortages are common in sub-Saharan Africa where approximately a 100 million people are malnourished. Drought and soils low in fertility, especially low nitrogen causes most of these food shortages. Less than 7% of agricultural land is irrigated and most of the maize is not well fertilized, as farmers are either too poor to afford fertilizers, or have no access to fertilizers, or are discouraged from using the fertilizers because of the risk of losing the crop to periodic droughts. Some countries in the region with low fertilizer application rates

are: Zimbabwe (43 kg/ha), Kenya (34 kg/ha), and Uganda (70 kg/ha) as compared with ideal rates of 200 kg/ha (Pingali 2001). Average maize yield is 1.3 t/ha, which is about 1 t/ha less than the average of all developing countries (CIMMYT-Zimbabwe 2000). Nitrogen Use Efficient varieties, are estimated to increase average maize yield from 1.2 to 1.5 t/ha and varieties selected for drought tolerance have also been observed to yield higher under low-nitrogen conditions (Pingali 2001).

Early maturing varieties (varieties that flower between 55 and 60 days, and reach physiological maturity at 120 days after emergence in the main season at Harare [1,500 m above sea level, latitude 17° S and longitude 31° E]) are planted to about 2.7 m ha in Eastern and Southern Africa and the area ranges from 0.2 m ha in Tanzanian Lowlands to as much as 1 m ha in Zimbabwe (CIMMYT-Zimbabwe 2000). The preference for growing early maturing maize varieties are that: (a) they are ideal for off-season plantings in riverbeds; (b) they provide an early harvest during the main season to bridge the "hungry" season before harvest of a full-season crop (important in areas where there are two growing seasons, like some parts of Kenya and Tanzania); (c) they can be used to produce a crop during the secondary short rain season, which enables the planting of a full season maize crop or other crops in the following main season; (d) they are ideal for intercropping as they provide less competition for moisture, light, and nutrients than later maturing varieties; and (e) they provide flexibility in planting dates, which enables multiple plantings in a season to spread risk of losing a single crop to drought and late plantings during delayed onset of rainfall; and (f) help escape terminal drought at the end of the cropping season (CIMMYT-Zimbabwe 2000).

Most of the current germplasm developed at CIMMYT-Zimbabwe (International Maize and Wheat Research Institute) are intermediate to late in maturity. Development of early-maturing drought and nitrogen stress tolerant maize germplasm is therefore one of the important programs CIMMYT is undertaking in order to alleviate hunger in sub-Saharan Africa. Since CIMMYT's early maturing maize breeding program is relatively young, there is little information on combining abilities of lines and hence there are no early maturing testers that would be appropriate for a program developing early maturing hybrids. The

hybrid products targeted for the region are three-way and double cross hybrids. Therefore the breeding programs in CIMMYT-Zimbabwe use (within heterotic group) single crosses as testers as they enable the identification of three-way and double cross hybrids during the process of early generation testing. Current testers in use are intermediate to late maturing. While these testers have proved to be extremely useful in deriving intermediate to late maturing hybrids, they limit the direct identification of early maturing hybrids. Therefore the objectives of this study were to determine heterotic relationships amongst 11 inbred lines and one open pollinated variety and to identify single-cross early maturing testers for use in three-way hybrid development.

Materials and methods

Eleven fixed inbred lines and one open-pollinated variety (OPV) (Table 1) were crossed in a diallel to give 66 crosses excluding reciprocal crosses. CIMMYT-Zimbabwe's early maturing maize breeding program was still new and hence there was limited choice of fixed inbred lines available for hybrid development. Therefore population ZEWAc1, an elite early maturing OPV being used as a tester for hybrid development, was included in the diallel. The diallel crosses were made in the winter of 2002 at Muzarabani, Zimbabwe. Each cross was made using paired plots of two rows each and 4 m length. In case of the OPV parent there were 20 rows planted to ensure that pollen from at least 200 plants were used for pollination. All the possible 66 crosses were made in both directions, using bulk pollen when using the OPV parent as male but making plant-to-plant crosses where crosses were made between inbred lines. Seeds of each cross and its reciprocal were bulked for use in the trials. Seed increase of the parents was done simultaneously in the same season.

The 66 crosses and 12 parents were evaluated in different trials in four environments in Zimbabwe during the summer of 2003 (optimal and managed low N) and winter of 2003 (managed drought). The parent trials were planted in the same block in close proximity to the hybrid trial and on the same planting date. Trials were planted at CIMMYT station (Harare) (1,480 masl, 17°43′ S latitude, 31°05′ E longitude, and 820 mm per annum rainfall), ART Farm (Harare)

Table 1 Pedigrees, heterotic groups and maturity of parental germplasm

Parent	Source germplasm	Germplasm type	Maturity	Principal characteristics
P1	CML395	Fixed inbred line	Late	GLS resistant, flint
P2	CML504	Fixed inbred line	Intermediate	Streak resistant derivative from a composite consisting of popular inbred lines
P3	K64R	Fixed inbred line	Early	Streak resistant version of a popular regional inbred line
P4	INTA	Fixed inbred line	Intermediate	Good combining ability
P5	Composite Early 20	Fixed inbred line	Early	Streak resistant line with good combining ability derived from a composite of regional early maturing lines
P6	Tropical early white synthetic	Fixed inbred line	Early	Streak resistant line with good combining ability derived from a drought tolerant composite of regional early maturing lines
P7	CML509	Fixed inbred line	Early	Drought tolerant inbred line with streak resistance and good combining ability
P8	CML505	Fixed inbred line	Early	Drought tolerant inbred line with streak resistance and good combining ability
P9	Tropical intermediate white synthetic	Fixed inbred line	Intermediate	Streak resistant derivative from a composite of early maturing regional lines and tropical lines
P10	Population 300	Fixed inbred line	Intermediate	Streak tolerant line derived from a tropically adapted population with downy mildew resistance
P11	Population 300	Fixed inbred line	Intermediate	Streak tolerant line derived from a tropically adapted population with downy mildew resistance
P12	ZEWAC1	Open pollinated variety	Early	Extra early maturing population developed from a wide pool of regionally developed inbred lines

(1,468 masl, 17°48′ S latitude, 31°05′ E longitude, and 890 mm per annum rainfall), Kadoma (1,155 masl, 18°32′ S latitude, 30°90′ E longitude, and 727 mm per annum rainfall), and Chiredzi (433 masl, 21°02′ S latitude, 31°58′ E longitude, and 300 mm per annum rainfall). Kadoma and ART Farm were under optimal conditions of fertilizers and moisture. At the CIMMYT Station (Harare), the trial was grown under nitrogen stress while that at Chiredzi Research Station the trial was grown under drought stress. The ART Farm, Harare CIMMYT station and Kadoma trials were planted during the summer of 2003 while the Chiredzi trial was planted during the dry season (winter) of 2003. The Harare site with managed low nitrogen was a block depleted of nitrogen by growing summer maize and irrigated winter wheat continuously for 6 years without any nitrogen applied to any crop. Drought at Chiredzi was managed through controlled irrigation. A total of 250 mm irrigation was all that was applied to the crop in the first 50 days of planting. This regime caused severe drought stress at flowering and grain filling time.

The experimental design used was an Alpha (0, 1) lattice with two replicates for each environment with six plots per incomplete block (giving 11 incomplete blocks in each replication). The experimental unit was one 4-m row spaced at 75 cm. Two seeds were planted per station spaced 25 cm between and thinned to one plant for each planting station to give a final planting density of 53,333 plants ha^{-1}. Data was recorded for grain yield (GY), moisture (MOI), anthesis date (AD) (number of days after planting when 50% of the plants shed pollen), silking date (SD) (number of days after planting when 50% of the plants extrude silk), anthesis silking interval (ASI) = SD − AD, ears per plant (EPP) (number of ears with at least one fully developed grain divided by the number of harvested plants), plant height (PH) (height between the base of a plant to the insertion of the first tassel branch of the same plant), ear height (EH) (height between the base of a plant to the insertion of the top ear of the same plant), senescence (SEN) (leaf senescence score on scale from 1 to 10 taken during grain-filling by estimating the percentage

of dead leaf area and dividing it by 10), husk cover (HC) (percentage of plants with ears that are not completely covered by the husks), grain texture (TEX) (rated on a scale from 1 (flint) to 5 (dent)), and root lodging (RL) (percentage of plants that show root lodging, i.e. those stems that are inclining by more than 45°). Shelled grain weight per plot was adjusted to 12.5% grain moisture and converted to tons per hectare. The ratio of ear height to plant height was the measure of ear position (EPO). A list of abbreviations used for the traits is presented in Table 2.

Statistical analysis

Analyses of variance were conducted for grain yield (GY), anthesis date (AD), silking date (SD), plant height (PH), ear height (EH), root lodging (RL), stem lodging (SL), bad husk cover (HC), senescence (SEN), texture (TEX), and ears per plant (EPP) using lattice adjusted means for each environment separately (data not shown) and then combined across significant environments. Environments and replications within environments were considered random and genotypes as fixed effects. Griffing´s (1956) method 4

(excluding parents and reciprocal F1s), model I (fixed model for parental effects) adapted to a SAS program (SAS 1997) for diallel analysis was used to analyze the data for general combining ability (GCA) and specific combining ability (SCA) effects. GCA and SCA effects for the different traits were calculated based on averages across 4, 3, or 2 sites. The model: $Y_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ijk}$; where Y_{ijk} is the observed measurement for the ijth cross grown in the kth replication or environment; μ is the population mean; g_i, and g_j are the GCA effects; s_{ij} the SCA effect; and e_{ijk} the error term associated with the ijth cross evaluated in the kth replication or environment. The restrictions imposed on the combining ability effects are: $\sum g_i = 0$, and $\sum s_{ij} = 0$ for each j (Griffing 1956).

Results and discussion

Yields, analysis of variance and combining ability analyses

Mean yields for the trials were 8.3 t/ha (optimum conditions), 3.2 t/ha (low nitrogen conditions) and

Table 2 List of trait abbreviations used and their description

Abbreviation	Trait	Units	Trait description
GY	Grain yield	Tons per ha (t/ha)	Shelled grain weight adjusted to 12.5% moisture and converted to tons per hectare
AD	Anthesis date	Days	Number of days after planting when 50% of the plants shed pollen
SD	Silking date	Days	Number of days after planting when 50% of the plants extrude silk
ASI	Anthesis silking interval	Days	SD − AD
PH	Plant height	cm	Height between the base of a plant to the insertion of the first tassel branch of the same plant
EH	Ear height	cm	Height between the base of a plant to the insertion of the top ear of the same plant
EPO	Ear position	0–1	Ratio of ear height to plant height
RL	Root lodging	%	Percentage of plants that show root lodging, i.e. those stems that are inclining by more than 45°
TEX	Grain texture	1–5	Rated on a scale from 1 (flint) to 5 (dent)
HC	Bad husk cover	%	Percentage of plants with ears that are not completely covered by the husks
SEN	Senescence	1–10	Leaf senescence score on scale from 1 to 10 taken during grain-filling by estimating the percentage of dead leaf area and dividing it by 10
EPP	Ears per plant	0–1	Number of ears with at least one fully developed grain divided by the number of harvested plants
MOI	Moisture	%	Percentage moisture as determined by the moisture meter

2.1 t/ha (drought conditions). The yields under low N and drought obtained in these trials were close to the stipulated limits recommended by Bolaños and Edmeades ([1996]) who determined that average yields under such abiotic stresses have to be between 20% and 30% of what the average yield would have been in the same location under optimum management to be able to select varieties that perform reasonably well both under optimum and stress conditions.

Significant differences ($P < 0.01$) due to environment were observed for all the nine traits presented in Table 3. This confirmed that each of the four targeted locations were unique as desired. Mean squares due to entries were significant ($P < 0.01$) for GY, PH and EPO at four sites; AD and ASI at three sites; and HC and TEX at two sites. HC and TEX were measured at the two optimal sites only, as cobs grown under stress are generally poorly filled and husk-cover problems and grain texture cannot be accurately recorded. The partitioning of entry mean squares into general combining ability (GCA) and specific combining ability (SCA) mean squares showed GCA mean squares to be significantly different ($P < 0.01$) for GY, PH, AD, TEX, HC and for EPO and ASI ($P < 0.05$). SCA mean squares were significantly different ($P < 0.01$) for PH, EPO, AD and HC. Significant differences for entry, GCA and SCA mean squares implied that there was scope for selecting for these traits among the entries, hence a chance to identify a potentially discriminating tester from a non-discriminating tester.

Across locations GCA sums of squares were larger than SCA sums of squares for GY (87%), AD (84%), TEX (72%) and PH (65%) while SCA sums of sums of squares were larger for ASI (52%), EPP (55%), HC (55%), RL (53%) and EPO (61%). The predominance of GCA sums of squares to SCA sums of squares for GY, AD, PH and TEX is an indication of the relative importance of additive gene action to non-additive gene action for these traits (Beck et al. [1990]). Additive genetic variance, an expression that is captured by the general combining ability of parents, is a factor highly emphasized during selection of testers. Thus for traits EPO, RL, ASI, EPP and HC which had a predominance of SCA sums of squares contributing to total sums of squares, the implication is that non-additive gene action is relatively more important for these traits.

Genotype (entry) by environment interaction

Entries (genotypes) by environments interactions were highly significant ($P < 0.01$) for all traits except HC (Table 3). Partitioning of entry by environment interactions into GCA by environment (GCA $\times$ E) and SCA by environment (SCA $\times$ E), showed that: (a) GCA $\times$ E was significant ($P < 0.05$) for all 9 traits; (b) SCA $\times$ E was significant ($P < 0.05$) for GY, ASI, RL and EPP. GCA $\times$ E interactions for the nine traits above is an indication of variation of general combining ability of lines under different environments. Of the hybrids, single crosses are more sensitive

Table 3 Combined analysis of variance of GY, PH and EPO (across four sites), AD, ASI (across 3 sites) and RL, EPP, TEX and HC (across 2 sites) in Zimbabwe

Source	df	GY t/ha MS	PH cm MS	EPO 0–1 MS	df	AD days MS	ASI days MS	df	RL% MS	EPP # MS	TEX 1–5 MS	HC % MS
Environment (Env)	3	714.5**	56039**	0.2166**	2	990.4**	147.2**	1	1895.5**	0.43**	8.7**	1276.0**
Entry	65	1.9**	568.5**	0.0054**	65	16.4**	5.9*	65	126.9	0.02	0.3**	61.4**
GCA	11	9.5**	2172.2**	0.0124*	11	81.0**	16.8*	11	352.4	0.04	1.3**	163.5**
SCA	54	0.9	111.5**	0.0017**	54	3.2**	3.6	54	81.0	0.01	0.1	40.6**
Env*Entry	195	0.9**	92.3**	0.0011**	195	2.0**	3.7**	195	84.9**	0.02**	0.1**	23.5
GCA*Env	33	1.8**	337.3**	0.0044**	22	3.1**	6.4**	11	166.9**	0.02*	0.2**	35.1*
SCA*Env	162	0.7**	42.4	0.0004	173	1.7	3.2**	184	68.2**	0.01*	0.1**	21.1
Residual	264	0.4	57.4	0.0008	198	1.3	1.6	132	23.2	0.01	0.1	18.6

MS: Mean square; df: degrees of freedom; GY: Grain yield; PH: Plant height; EPO: Ear position; AD: Anthesis date; ASI: Anthesis silking interval; RL: Root lodging; EPP: Ears per plant; TEX: Texture; HC: Bad husk cover

* Significant at $P = 0.05$; ** significant at $P = 0.01$

to environments (Hallauer and Miranda 1988) than three-way or double crosses. In addition, stress environments produce high genotype by environment interactions (Bänziger et al. 2000). ASI increased under stress (0.45 days optimal, 2.9 days drought and 3.6 low N). AD increases under cooler temperatures, while decreasing under hot temperatures, because growth rates are slower under cooler temperatures compared to hot temperatures (mean AD was 69.2 days under summer conditions at ART farm Harare and 98.6 days at Chiredzi in the cool season). GCA for grain yield (and hence heritability) increases under stress especially drought stress (Betrán et al. 2003). Significant SCA × E interaction of GY, ASI, RL and EPP mean that specific hybrids differed in the way they expressed these traits under different environments. Testing lines under different environments ensures selection of stable testers i.e. those which perform to the potential of the environment always. Scott (1967) demonstrated that selection for such stable genotypes was possible.

GCA and SCA effects

Eight lines: P1, P2, P4, P7, P8, P9, P10 and P11 had positive GCA effects for GY (Table 4). The early maturing OPV, ZEWA (P12) together with lines P3

and P6 had very low GCA effects for GY (−0.14, −0.80 and −0.66 t/ha, respectively). GCA effects for anthesis date (AD) for P3, P5, P6 and P12 were −3.0, −1.6, −1.9 and −2.2 days, respectively. Although P1 (CML395), had the biggest GCA effect value for AD (3.7 days) (late maturing) it was not the best for GCA effects for grain yield. Maturity and yield are positively correlated. It is common knowledge among breeders that an earlier maturing variety, owing to its shorter life cycle, is predisposed to lower yields than a later maturing variety which has the opportunity to draw on nutrients and photosynthesize over a longer period. It is a common experience of maize breeders that merely selecting for higher yields in populations (without exercising selection for maturity) tends to make them later in maturity while selecting only for earliness (without selecting for higher yield) makes the population lower yielding. Therefore while selecting early maturing germplasm maintaining a balance between earlier maturity and higher yield is critical.

Significant differences were observed among the lines for EPP, an important secondary trait used to select drought tolerant germplasm. The highest positive value for EPP was seen in P9 (0.08). Lines with poorest per se values for GCA effects for SEN were those with positive values: lines P10 (0.03), P12 (0.16), P8 (0.24), P5 (0.25), P2 (0.29), P6 (0.29) and

Table 4 GCA Effects of parental lines for different traits

Parent	GY (t/ha)	SEN (1–10)	AD (days)	ASI (days)	PH (cm)	EH (cm)	EPO (0–1)	RL (%)	EPP (#)	HC (%)	TEX (1–5)
P7	0.49	−0.42	−1.0	0.4	4.2	2.2	0.001	−3.8	−0.02	−1.5	0.3
P9	0.45	−0.15	0.9	−0.6	2.7	5.1	0.020	1.3	0.08	0.9	0.1
P2	0.41	0.29	1.4	0.4	8.1	5.1	0.003	−0.7	−0.02	−0.7	0.1
P1	0.24	−0.41	3.7	0.9	13.7	10.3	0.018	7.2	−0.05	−1.3	0.1
P10	0.21	0.03	2.4	−0.2	9.1	13.2	0.043	−0.2	0.01	0.7	−0.1
P11	0.13	0.49	1.6	−0.2	0.5	−1.6	−0.012	−0.3	0.03	−4.4	−0.1
P4	0.06	−0.14	0.4	0.8	2.2	−0.8	−0.006	3.9	−0.02	1.4	0.2
P8	0.00	0.24	−0.8	−0.6	−13.5	−2.9	0.020	−2.7	0.03	−0.9	0.3
P12	−0.14	0.16	−2.2	−0.1	−5.5	−4.8	−0.009	−2.1	−0.02	0.4	0.1
P5	−0.38	0.25	−1.6	0.2	−10.2	−12.3	−0.039	−3.2	0.00	4.1	−0.3
P6	−0.66	0.29	−1.9	−1.1	−2.4	−3.4	−0.012	−3.7	0.02	1.7	−0.1
P3	−0.80	−0.61	−3.0	0.0	−8.8	−10.0	−0.029	4.4	−0.04	−0.4	−0.6
MEAN	0.00	0.00	0.0	0.0	0.0	0.0	0.000	0.0	0.00	0.0	0.0
LSD	0.39	1.10	1.0	0.8	5.5	4.9	0.020	2.0	0.02	2.0	0.4

Key—SEN: Senescence (scale 1–10); PH: Plant height (cm); GY: Grain yield (t/ha); ASI: Anthesis-silking interval (days); EH: Ear height (cm); AD: Anthesis date (days); EPP: Ears per plant (count); RL: Root lodging (%); EPO: Ear position (0–1); TEX:Grain texture (scale 1–5); HC: Husk cover (%)

P11 (0.49). Positive values are an indication of rapid senescence while negative values indicate slow senescence. The best line, considering the SCA effects for SEN was P3 (−0.61) (Table 4). There were significant differences for GCA effects for ASI and ranged from −1.1 days to 0.9 days (Table 4). Stress intolerant varieties tend to have larger ASIs under drought and low N stress than varieties that are tolerant to these abiotic stresses. P1 had highest GCA effects for PH (13.74 cm) and P8 had the smallest and hence a desirable GCA effect for PH (−13.46 cm) as shorter plants are less prone to lodging (Table 4). There was variation for GCA effects for EPO among the lines. P5 was the line with the least GCA effect for EPO (−0.04). The line with the largest GCA effect for EPO was P10 (0.04). Lower ear position is desired as plants with lower ear position tend to lodge less. GCA effects for EH showed significant variation among lines where P10 had the largest effects (13.19 cm) (least desirable) and P5 was the best (−12.33 cm). GCA effects for RL showed significant variation among lines. P1 was the poorest for this trait (7.2%) while P7 was the best (−3.85%). There was very little variation in GCA effects for TEX among the twelve parents.

There was significant variation for AD, PH, EPO and HC among the crosses which implies that selection based on SCA effects could be used to pick good hybrids. Largest SCA effects for AD were 3.0 days and the smallest −1.4 days. PH effects ranged from −12.2 cm to 9.8 cm. SCA effects for EPO ranged from −0.07 cm to 0.02 cm while that for HC ranged from −4.2% to 15.0%.

Heterotic relationships

P1 and P5 were used to classify the lines into heterotic groups as the cross P1/P5 showed the maximum SCA effect (0.76 t/ha). Positive SCA effects indicate that lines are in opposite heterotic groups while negative SCA effects indicate that lines are in the same heterotic group (Vasal et al. 1992). From previous classifications, it is known that P5 belongs to group A and P1 belongs to group B. Based on SCA effects (Table 5) and the absolute value of the difference between SCA effects of a parents cross with P1 and P5 (Table 6), lines P4 and P6 could be classified as group B while line P7 could be classified as group A. The SCA effect of P1/P8 was positive (0.18 t/ha), and that of P5/P8 was negative (−0.38 t/ha). The absolute difference between SCA effects of P1/P8 and P5/P8 was 0.6 t/ha (Table 6). Although the SCA effects were non-significant for P1/P8 and P5/P8, the absolute difference between the SCA effects between them was closer to the LSD, implying that P8 had a tendency to be heterotically similar to group A. In spite of this lack of clarity it is suggested that P8 be classified into group A as there is supporting evidence from early generation tests (data not shown) that P8 belongs to heterotic group A. There was a lack of distinctness of the other lines with respect to their heterotic patterns as the differences were not significant. For instance, lines P2 and P10 had negative SCA effects for GY with both testers perhaps indicating a need to identify more testers and classify more heterotic groups than currently used. On the same note, line P9 could

Table 5 SCA effects of grain yield in t/ha of 66 hybrids from the 12 parent diallel

	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
P1	−0.01	−0.05	−0.88	0.76	−0.89	0.39	0.18	0.28	−0.01	0.12	−0.27
P2		0.21	0.00	−0.18	−0.41	0.03	0.64	−0.13	0.29	0.00	−0.44
P3			0.60	0.44	−0.36	−0.11	0.41	−0.22	−0.41	−0.44	−0.07
P4				−0.06	−0.14	0.11	0.26	−0.61	−0.16	0.16	0.71
P5					0.03	−0.85	−0.38	0.53	−0.32	−0.19	0.21
P6						0.35	0.26	0.10	−0.07	0.61	0.51
P7							0.47	0.24	0.07	0.00	−0.69
P8								−0.87	−0.43	−0.22	−0.31
P9									0.61	−0.20	0.27
P10										0.43	0.00
P11											0.10

LSD = 0.77

Table 6 Absolute values of the differences of SCA effects (t/ha) between hybrids of a parent with P1 and P5

	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
P1	−0.01	−0.05	−0.88	0.76	−0.89	0.39	0.18	0.28	−0.01	0.12	−0.27
P5	−0.18	0.44	−0.06		0.03	−0.85	−0.38	0.53	−0.32	−0.19	0.21
Absolute value of the difference between P1 and P5	0.17	0.49	0.81		0.93	1.24	0.56	0.26	0.32	0.31	0.48
LSD	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77

potentially be classified into group AB, because it had positive SCA effects for GY with both testers. Warburton et al. (2002) recommended classifying a line into a new group if it has positive SCA effects with both testers. Using the absolute values of the differences between SCA effects of a line with opposing testers as a measure of heterotic tendencies, line P11 had a tendency to be classified into group A. Likewise line P3 had a tendency to be heterotically similar to group B, in conformation with previous studies. P12 thought to be heterotic group A shows negative SCA effect with P1 (group B) and a positive effect with P5 (group A). Such seeming changes in heterotic behaviour are not unusual as combining abilities are specific to the group of parents being tested. Also lines belonging to the same heterotic group may not have absolutely identical heterotic patterns because of small differences in the alleles they may be carrying (Rawlings and Thompson 1962). Heterotic groups are not absolute as reported by Hallauer and Miranda (1988) and thus a changing or reclassification of lines into groups may be necessary. Heterotic groups A and B at CIMMYT have been aligned similar to some of the well known heterotic patterns across the globe, viz. Tuxpeño vs. ETO Blanco of Mexico, Reid Yellow Dent vs Lancaster of the US, Kitale vs. Ecuador of the east-African highlands and N3 vs SC of southern Africa. Thus, group A is thought to exhibit heterosis similar to N3, Tuxpeño, Kitale and Reid. Group B is thought to be exhibit heterosis similar to SC, ETO Blanco, Ecuador and Lancaster (Mickelson et al. 2001). Materials defined as being in group AB have high heterosis with both groups A and B germplasm. For a breeding program geared towards development of three-way hybrids, this presents more options where hybrids could be developed using all three heterotic groups (e.g. A × B single cross crossed to a line from group AB) without having to deal with the inbreeding depression of the female seed parent that

one might encounter when an intra-group cross is made to form the seed parent.

Tester identification

The choice of potential lines as testers was based on (a) display of positive GCA effects, (b) classification into heterotic groups and (c) per se grain yield. Based on this, only five lines qualified as potential testers. From group B: P4 and P6; and from group A: P7, P8 and P11 as they were defined into heterotic groups and gave reasonable per se yields under different environments (Table 7). Further, the choice of a potential single cross tester amongst these lines was based on (a) display of reasonably good GCA effects of the inbred lines constituting the single cross; (b) grouping of the inbred lines constituting the single cross to the same heterotic group; (c) reasonable per se performance of the inbred lines constituting the single cross; (d) a reasonably good yielding ability of the potential single cross tester to qualify its use as a female seed parent in successful three-way and double-cross hybrids, posttesting. With the given short list of potential inbred lines only three single combinations were possible for group A (P7/P8, P7/P11, P8/P11) and only one combination for group B (P4/P6).

P7 had the highest GCA effects for GY (0.49 t/ha) among group A lines (P7, P8 and P11) (Table 4). P7 had good per se grain yield under optimal conditions (3.6 t/ha) (Table 7). SCA effects of P11 were not significantly different from zero in several crosses in the trials (data not shown) while hybrids with P1 and P5 were. This is an indication of inefficiency in discriminating lines by P11. Although line P8 had lower GCA effects compared to line P11, it was selected instead of P11 for the following reasons: (a) AD for the cross P7/P8 (64.4 days) is earlier than crosses where P11 is involved (67.4 days (P7/P11); 67.5 days (P8/P11) (Table 7); (b) SCA effects for GY for crosses P8/P11

Table 7 Grain yield, SCA effects and GCA effects for yield of 15 selected hybrids (the best 12 and the worst 12 based on SCA effects and three selected hybrids with low SCA)

Hybrid	Optimal			Low N			Drought			AD(days)	SCA effects (GY) t/ha	GCA effects (GY, t/ha)	
	GY t/ha	LPYld t/ha	HPYld t/ha	GY t/ha	LPYld t/ha	HPYld t/ha	GY t/ha	LPYld t/ha	HPYld Yld t/ha			P1	P2
P1/P5	9.4	4.0	5.8	3.2	2.1	3.3	2.2	1.2	3.2	68.2	0.76	0.24	−0.38
P4/P12	9.5	3.0	7.7	4.0	1.1	5.7	1.5	0.1	2.9	65.0	0.71	0.06	−0.14
P2/P8	10.5	3.4	4.0	3.0	0.6	1.6	2.0	0.2	1.1	68.1	0.64	0.41	0.00
P6/P11	8.2	1.6	3.2	3.3	0.8	3.3	2.5	0.0	0.3	67.8	0.61	−0.66	0.13
P9/P10	10.8	2.0	2.7	2.5	0.4	0.8	2.5	0.1	0.7	68.9	0.61	0.45	0.21
P3/P4	7.7	2.0	2.3	3.3	0.7	1.1	2.6	0.1	0.7	64.3	0.60	−0.80	0.06
P5/P9	8.6	2.7	2.9	4.0	0.8	1.1	2.9	0.7	1.2	65.5	0.53	−0.38	0.45
P6/P12	7.6	1.6	7.2	3.7	0.8	5.7	1.9	0.0	2.9	66.7	0.51	−0.66	−0.14
P7/P8	9.8	3.6	4.0	3.4	0.6	1.7	2.1	0.8	1.1	64.4	0.47	0.49	0.00
P3/P5	7.0	2.0	2.9	2.2	0.7	1.1	2.6	0.7	1.2	62.9	0.44	−0.80	−0.38
P10/P11	10.3	2.0	3.2	2.6	0.4	3.3	1.9	0.2	0.3	72.0	0.43	0.21	0.13
P3/P8	7.5	2.0	4.0	3.2	0.6	0.7	2.4	0.7	1.1	64.9	0.41	−0.80	0.00
P7/P11	9.5	3.2	3.5	3.7	1.7	3.3	1.9	0.3	0.8	67.4	0.00	0.49	0.13
P4/P6	7.8	1.6	2.3	2.7	0.8	1.1	0.8	0.0	0.1	65.6	−0.14	0.06	−0.66
P8/P11	8.3	3.2	3.9	3.2	0.6	3.3	1.8	0.3	1.1	67.5	−0.22	0.00	0.13
P5/P8	7.1	2.9	3.9	3.1	0.6	1.1	1.9	1.1	1.2	64.5	−0.38	−0.38	0.00
P3/P10	7.0	2.0	2.0	2.0	0.4	0.7	2.1	0.2	0.7	67.6	−0.41	−0.80	0.21
P2/P6	7.4	1.6	3.4	2.7	0.8	1.6	1.7	0.0	0.2	66.5	−0.41	0.41	−0.66
P8/P10	7.6	2.0	3.9	3.3	0.4	0.6	2.4	0.2	1.1	65.3	−0.43	0.00	0.21
P3/P11	6.6	2.0	3.2	2.6	0.7	3.3	1.4	0.3	0.7	66.3	−0.44	−0.80	0.13
P2/P12	8.1	3.4	7.2	3.8	1.6	5.7	1.4	0.2	2.9	66.3	−0.44	0.41	−0.14
P4/P9	8.3	2.3	2.7	2.7	0.8	1.1	1.7	0.1	0.7	67.0	−0.61	0.06	0.45
P7/P12	7.8	3.5	7.2	2.7	1.7	5.7	2.0	0.8	2.9	64.1	−0.69	0.49	−0.14
P5/P7	7.2	2.9	3.5	2.3	1.1	1.7	1.9	0.8	1.2	64.1	−0.85	−0.38	0.49
P8/P9	8.0	2.7	3.9	2.5	0.6	0.8	1.9	0.7	1.1	66.7	−0.87	0.00	0.45
P1/P4	7.6	2.3	2.3	3.7	1.1	3.3	0.6	0.1	3.2	68.4	−0.88	0.24	0.06
P1/P6	6.6	1.6	1.6	2.5	0.8	3.3	1.2	0.0	3.2	67.6	−0.89	0.24	−0.66
Mean	8.3	2.5	3.9	3.2	0.9	2.6	2.1	0.4	1.5	66.4	0.00	0.00	0.00
LSD	1.7			2.5			0.9			2.4	0.77	0.39	0.39

Key—GY: Grain yield; HPYld: High parent yield; AD: Anthesis date; LPYld: Low parent yield

(−0.22 t/ha) and P7/P11 (0.00 t/ha) were lower compared to P7/P8 (0.47 t/ha); (c) line P8 had higher GCA effects for most traits: SEN, AD, ASI, PH, EH and RL compared to line P11; (d) yields of crosses with P11 were less than yield of cross P7/P8; (e) P8 had SCA effects significantly different from zero with many lines in the trials implying it was better at discriminating lines than P11; (f) P8 had good per se grain yield under optimal conditions (4 t/ha) (Table 7) and (g) heterotic classification of P11 was not clear.

Even though one would expect negative SCA effects of crosses from lines of the same heterotic group sufficient variation exists within heterotic groups of CIMMYT germplasm (again perhaps indicating that groupings can be further refined). Such heterosis translates into high yields which could be attributed to the high yields that single cross P7/P8 gave under optimum (9.8 t/ha) and drought (3.4 t/ha) conditions compared to the other potential testers. Yield of the single cross should be high enough to

justify its use as the female seed parent in successful crosses, while at the same time the yield should not be indicative of having completely exploited the available dominance amongst lines. In general, tropical maize germplasm is known to have an intra-group diversity (Han et al. 1991) that is sufficient to exploit the heterosis for seed production in three-way and double cross combinations. Yield of P7/P8 under low nitrogen was similar to the other potential testers. High yields under drought conditions could be explained by the low rate of senescence (SEN) (−0.34) of P7/P8 (Table 4). Such a low rate of senescence prolongs the time for grain filling. Also related to high yields under drought could be the high GCA effect for EPP coming from P8 (Table 4). Susceptible genotypes tend to be barren under drought. Good yields from this single cross especially under drought would ensure reasonable seed yields. Seed production plots on farmers fields in southern Africa are prone to drought, resulting in reduced seed yields, thus drought tolerance in a seed parent is desirable. P7 and P8 had crossover interaction (i.e. switched ranks) in the low N and drought environments: Line P7 (1.7 t/ha) was better than line P8 (0.6 t/ha) under low N, while line P8 (1.1 t/ha) was better than line P7 (0.8 t/ha) under drought. This might make this single cross well buffered, thus enabling it to perform as a seed parent under diverse environments and also passing on its stress tolerance to the product being made.

Perhaps some of these plausible mechanisms suggested contribute to the higher yield and heterosis of the within heterotic group crosses by largely exploiting additive variance while retaining the dominance effects to be fully exploited in the final across heterotic group three-way or double cross hybrid. The next set of experiments should be targeted at confirming these results perhaps through a North Carolina Design II type study where P7/P8 and the intermediate to late maturing testers currently being used in the program, are crossed to a series of inbred lines. Also, the experiments need to be evaluated in a wider range of environments to have a better representation of the target zone and confirm the stability of the tester.

The single cross from group B, P4/P6 was not suitable as a tester because of low GCA effects of both lines P4 (0.06 t/ha) and P6 (−0.66 t/ha). Also most crosses made from these lines had negative SCA effects for GY (Table 7). Line 1 (CML 395) currently being used as a tester in the breeding program and used as a benchmark in this study, is late maturing. Further work needs to be done to identify an early maturing tester for group B.

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