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Validation of the use of secondary traits and selection indices for drought tolerance in tropical maize (*Zea mays* L.)

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Breeding progress for drought tolerance in maize has been slow since drought tolerance is a complex trait controlled by many genes. Breeders improving maize for drought tolerance have therefore been using secondary traits and selection indices for selecting the best genotypes under drought stress. The objectives of this study were to determine the combining ability of the inbred lines in stress and non stress environments as well as compare the use and efficacy of secondary traits and selection indices in selecting for drought tolerant genotypes. Fifty hybrids formed through a North Carolina Design II and four checks were evaluated using the 0.1 alpha lattice planting design under optimum and drought environments. General combining ability (GCA), specific combining ability (SCA) and seven drought indices (SDI), stress susceptibility index (SSI), tolerance (TOL), yield index (YI), yield stability index (YSI), mean productivity index (MPI), stress tolerance index (STI) and geometric productivity index (GMP) were used in the computation of results. Results showed that under drought conditions, GCA was highly significant ($P < 0.001$) for grain yield, anthesis-silking interval (ASI) and ears per plant (EPP) while SCA was significant for grain yield and EPP. Selection indices STI and GMP had positive and significant correlation ($P < 0.01$) with grain yield under both drought stress (Y_s) and optimum (Y_p) conditions. EPP also had significant ($P < 0.01$) and positive correlation with Y_s and Y_p . ASI had significant and negative correlation with Y_s ($P < 0.01$) and TOL ($P < 0.05$). In addition EPP had a positive and significant correlation with STI ($P < 0.05$) and GMP ($P < 0.01$). The results indicated that ASI, EPP, STI and GMP are effective in identifying high yielding genotypes under different moisture regimes. Narrow sense heritability showed that phenotypic variation attributed to genetic effects increased under stress conditions for EPP and ASI and making them more reliable parameters for use in selecting for genotypes under stress conditions. The two secondary traits (ASI and EPP) together with two drought indices; (STI and GMP) can therefore be used in tandem for increased efficiency in selecting for genotypes under stress environments.

Key words: Combining ability drought stress, drought index, heritability.

INTRODUCTION

Globally, 160 million ha of maize is under rainfed conditions and annual yield losses to drought are estimated at around 25% (Edmeades, 2008). The losses are greater in subtropical countries that rely on an erratic

and unpredictable rainfall. Future projections indicate that maize production will face a reduction in irrigation volumes, even in regions where supplemental water is essential for securing a profitable harvest (Rosegrant et al., 2002). This is attributed to climatic change and the general global warming culminating in less average rainfall in most regions. Although appropriate irrigation and/or other agronomic practices may mitigate the reduction in yield caused by drought and their effects

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Table 1. Parental material and selection criteria used in testcross development.

Line	Selection criteria	Tester	Selection criteria
[CML445/ZM621B]-2-1-2-3-1-B*8	MSV and drought	CML312	MSV and drought
CML312/[TUXPSEQ]C1F2/P49-SR]F2-45-3-2-1-BB	MSV and drought	CML442	MSV and drought
MSRXPOOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-B	Drought	CML537	MSV and drought
TS6C1F238-1-3-3-1-2-#-BB	MSV and drought	CML538	MSV and drought
P501SRc0-F2-47-3-1-1-BB	MSV and drought	CKL5005	MSV
ZM521B-66-4-1-1-B*5	MSV and drought		
SYN-USAB2/SYN-ELIB2]-12-1-1-1-B*5	MSV		
SYN-USAB2-ELIB2]-35-2-3-1-B*4	MSV		
Z97SYNGLS(B)-F2-188-2-1-3-B*4	MSV and drought		
MAS[206/312]-23-2-1-3-B*5	MSV and drought		

largely depend on the genetic make-up of the crop. This therefore means that breeders in stress prone environments will need to double their efforts on breeding for drought tolerance.

Low heritability for drought tolerance and lack of effective selection approaches limit development of genotypes that are tolerant to water stress. Grain yield is a complex trait controlled by several interacting genotypic and environmental factors. However, in maize there are yield components which are less complex, highly heritable and less influenced by environmental effects. The exploitation of these highly heritable components which are highly correlated to grain yield is therefore a more effective option than direct selection of yield *per se* (Kashiani and Saleh, 2010). By utilizing genetic correlations between traits, secondary traits can be used to improve primary traits that have low heritability or are difficult to measure (Malosetti et al., 2008). In the process correlation analysis can be used to determine efficacy or efficiency. Correlation analysis exploits the degree of association among important quantitative traits (Malik et al., 2005). The correlation coefficient analysis is useful in selection of several traits simultaneously influencing yield (Menkir, 2008). Selection for high yield potential entails genetically correlating yields components cultivars grown in two contrasting environments of one stressed and the other well watered.

A significant and positive genetic correlation of grain between stress and non stress sites will mean effective selection for drought tolerance while if correlation is zero or negative selection for grain yield alone will not be effective under drought. To evaluate response to drought stress, some selection indices based on mathematical relations between optimum and stress conditions have been developed. The interrelationship between yield and its contributing components can significantly improve the efficiency of crop breeding programs through the use of proper selection indices (Mohammadi et al., 2001). Research on maize by Khalili et al. (2004) showed that geometric mean productivity (GMP) and stability tolerance index (STI) can be used in selecting genotypes

with high yield in both stress and non stress environments. Direct selection of yield is often deceptive as it is highly influenced by environmental components (Tabeli et al., 2007). Thus this study aimed at assessing the reliability of selection indices and secondary traits and how they relate to the traditional parameters such as combining ability in an effort to improve selection efficiency under stress environments.

MATERIALS AND METHODS

Germplasm and experimental design

Fifteen CIMMYT white grained tropical inbred lines (10 lines and 5 testers) were crossed using the North Carolina Design II. The fifty resultant single cross hybrids, their parental lines and four hybrid checks were evaluated across six sites using the alpha 0.1 lattice design. The trial set was planted under optimum conditions at agricultural research trust farm (ART Farm; 31°E 17.13°S), Rattray Arnold Research Station (RARS; 31.5°E 17.43°S) and Kadoma Research Station (30.9°E 18.32°S). Drought stress evaluations were done off season, during summer and winter at Chiredzi (31.5°E 21.02°S) and winter only at Chisumbanje (33°E 22.1°S). The testcross evaluation was done using the alpha (0.1) lattice design. At each site trials were replicated twice, with each entry being planted in one row plots 5 m long, 75 cm inter-row spacing and 25 cm in-row spacing. Two seeds were planted per station and later thinned to give a plant population of 53000 plants/ha. The hybrid SC727 was used as drought susceptible check. The parental lines used in the study and the primary criteria for selections are shown in Table 1.

Optimum evaluation and stress management of the trials

Hybrids and their parents were evaluated separately in two trials planted adjacent to each other in the 6 sites (Table 2). The 2010B season is the dry cold season where managed drought stress was done using irrigation and withdrawing water for six weeks bracketing the flowering period. Parent inbred lines were also planted in the same environment as the test hybrids. In all the trials raw data for flowering dates (at 50% anthesis and 50% silking), plant and ear height, plant root and shoot standability, leaf senescence, disease and normalized difference vegetative index scores and grain weight were recorded. Some derived traits such as anthesis-silking interval (ASI), lodging percentage, ears per plant

Table 2. Characteristics of Experimental environments for cultigen evaluation.

Environment	Location	Season	Type of environment	Average grain yield (t/ha)	
				Hybrid	Inbreds
1	ART Harare	2010A	Optimum	8.77±0.85	3.89±0.68
2	RARS Harare	2010A	Optimum	8.72±0.34	4.00±1.03
3	Kadoma	2010A	Optimum	7.18±0.72	2.94±0.42
4	Chiredzi	2010A	Drought	2.17±0.23	1.03±0.54*
5	Chisumbanje	2010B	Drought	3.94±1.59	1.21±0.19
6	Chiredzi	2010B	Drought	2.90±0.75	0.71±0.23

*Random drought stress with stress simulating a managed drought site conditions as described by Banziger et al., 2000.

(EPP) and yield per hectare (at 12.5% moisture adjustment) were also calculated. The sixth site, Chiredzi 2010A site was considered a random drought site because it received less than 60% of its normal average of 425 mm and the drought stress coincided with the flowering and grain filling periods. The general drought screening was done following the evaluation procedure by Banziger et al. (2000).

Statistical analysis

Individual analyses of variance were computed for each trial using the PROC MIXED procedure from SAS (SAS, 2009) with hybrids and inbreds being considered as fixed effects, while replications and blocks were considered random effects. However, this study reports on the across site analyses of the data in the two environments. The adjusted means were used to estimate GCA and SCA effects, while heritability was calculated as the proportion of genetic variance over the total phenotypic variance.

Statistical analysis formulae

Drought tolerance indices were calculated using the following formulae:

Yield stability index (YSI)
 $YSI = Y_{si} / Y_{pi}$ (Lin et al., 1986).....(i)

Stress susceptibility index (SSI)
 $SSI = [1 - YSI] / SI$ (Fischer and Maurer, 1978).....(ii)

Yield index (YI)
 $YI = Y_{si} / Y_s$ (Gavuzzi et al., 1997).....(iii)

Stress tolerance index (STI)
 $STI = (Y_{pi} \times Y_{si}) / Y_p^2$ (Fernandez, 1992).....(iv)

Geometric mean productivity (GMP)
 $GMP = \sqrt{Y_{pi} \times Y_{si}}$ (Fernandez, 1992).....(v)

Tolerance index (TOL)
 $TOL = Y_{pi} - Y_{si}$ (Hossain et al., 1990)..... (vi)

Mean productivity (MP)
 $MP = (Y_{pi} + Y_{si}) / 2$ (Hossain et al., 1990).....(vii)

Stress intensity (SI)
 $SI = 1 - (Y_s / Y_p)$(viii)
 Where;

Y_{si} = yield of cultivar under stress condition
 Y_{pi} = yield of cultivar under optimum condition
 Y_s = total mean yield under stress condition
 Y_p = total mean yield under optimum condition

RESULTS

General combining ability of lines and testers

Combined analysis of optimum environments showed that there were significant line (female), tester (male), line x tester, line x environment and line x tester x environment effects for grain yield (Table 3). There were also significant differences for all traits across the 3 environments. SCA effects were also significant ($P < 0.001$) for all traits but senescence. Genotype x environment interactions, were shown for GY, PH and ET. Significant additive maternal effects were also recorded for GY, ASI, EPP, PH and ET while additive paternal effects were observed in GY, SEN, PH and ET. Maternal and paternal effects interactions with the environment were significant for GY, ASI, ET and EPP, PH respectively.

The analysis of variance across drought sites also showed significant differences among the testers, lines, line x tester, tester x environment and lines x environment for GY and ASI (Table 4). Significant effects were shown for GY, EPP and PH across the environments. Both additive maternal and paternal effects were significant for all traits except SEN. Significant interactions with the environment were recorded for GCA_m , GCA_f and SCA for GY and ASI.

Table 5 shows the SCA effects for GY, where Line 10 and Tester 5 had the highest SCA effect value of 1.603. Line 10 and tester 3 had the lowest yield expectation with an SCA value of -2.796. Line 5 recorded the most positive and significant SCA combination effects. Line 10 had the most negative and significant SCA effects.

The GCA effects for GY and the main secondary traits under stress indicate that line 2 was the best parent lines. Line 2 had good and significant GCA effects for GY, negative significant ASI effects which are ideal under

Table 3. Combined anova of three optimum sites for grain yield and agronomic traits.

Source of variation	DF	GY	ASI	SEN	EPP	PH	ET
Environ	2	153.7***	134.6***	49.54***	29.82***	8237.3***	24.36***
Rep/environ	3	0.26 ^{ns}	0.24 ^{ns}	8.76**	0.41 ^{ns}	1584.2***	2.12**
GCA _m	4	7.47***	0.43 ^{ns}	1.86**	1.93 ^{ns}	1990.7***	4.49**
GCA _f	9	8.06***	3.47**	0.51 ^{ns}	2.32**	1665.7***	2.51**
SCA	36	9.64***	1.94***	0.66 ^{ns}	2.94***	765.4***	0.52*
GCA _m * environ	8	2.21 ^{ns}	1.23 ^{ns}	1.16 ^{ns}	1.77*	301.9*	0.43 ^{ns}
GCA _f * environ	18	2.24*	3.11***	0.83 ^{ns}	1.13 ^{ns}	216.6 ^{ns}	0.51*
SCA * environ	72	1.91*	1.47 ^{ns}	0.81 ^{ns}	1.16 ^{ns}	725***	0.58**
Error	147						

*P<0.05; **P<0.01; ***P<0.001; ns = not significant. GY = Grain yield; ASI = Anthesis silking interval; SEN = Leaf senescence; EPP = Ears per plant; PH = Plant height; ET = Turcicum leaf blight.

Table 4. Combined ANOVA of three drought sites for grain yield and agronomic traits.

Source of variation	DF	GY	ASI	SEN	EPP	PH
Environment	2	153.76***	0.87 ^{ns}	0.28 ^{ns}	1.25***	2786.51***
Rep/environ	3	0.26 ^{ns}	0.62 ^{ns}	0.58 ^{ns}	0.007 ^{ns}	30.31 ^{ns}
GCA _m	4	8.21***	3.49***	0.96 ^{ns}	0.19***	649.75***
GCA _f	9	5.15***	2.27**	0.37 ^{ns}	0.11***	1147.42***
SCA	36	2.97***	1.30**	0.99 ^{ns}	0.12***	718.96***
GCA _m * environ	8	3.57***	2.69***		0.04 ^{ns}	255.65 ^{ns}
GCA _f * environ	18	5.79***	3.40***		0.06 ^{ns}	273.92 ^{ns}
SCA * environ	72	2.59**	1.21**		0.04 ^{ns}	360.51*
Error	147					

P<0.05; **P<0.01; ***P<0.001 ; ns = not significant. GY = Grain yield; ASI = Anthesis silking interval; SEN = Leaf senescence; EPP = Ears per plant; PH = Plant height.

Table 5. Combined analysis of drought SCA effects for grain yield.

Tester	Line									
	1	2	3	4	5	6	7	8	9	10
1	-0.024	-1.028	-0.902	0.470	0.469	0.716	0.319	0.178	1.035	-1.221
2	0.452	0.098	0.356	-0.799	0.375	-0.256	-0.188	-0.436	0.386	0.010
3	0.942	-0.572	0.774	0.449	0.395	0.342	0.452	-0.313	-0.272	-2.796
4	-0.284	0.727	0.352	-0.688	0.259	-0.382	0.371	1.365	-0.898	-0.807
5	-0.394	-0.083	-0.223	-0.476	-0.270	0.768	0.038	-1.097	0.634	1.603
LSD(0.05)	0.337	0.337	0.337	0.337	0.337	0.337	0.337	0.337	0.337	0.337

stress a positive EPP and negative EPO effects for a good ear placement. The worst line was line 8 which had the lowest GY GCA effects and insignificant favorable GCA effects for ASI, EPP and SEN (Table 6).

Table 7 shows the heritability estimates of grain yield and the secondary traits measured. The trend shows that generally heritability increased with drought stress for

most of the traits measured. Ears per plant, anthesis-silking interval and senescence had increased heritability under drought conditions. As expected grain yield and plant height had reduced heritability under moisture stress conditions. There was no maize leaf blight disease was not scored in the drought trials.

STI, GMP and MP have significant (P<0.01) and

Table 6. Combined line GCA analysis of drought sites for GY and secondary traits.

Line	GY	ASI	PH	EH	EPO	EPP	SEN
1	-0.250	-0.304	6.242	0.982	-0.013	-0.018	0.056
2	0.691	-0.402	2.697	-2.938	-0.021	0.020	0.118
3	0.131	0.148	-4.428	-0.188	0.013	-0.068	-0.127
4	-0.036	-0.145	-6.826	-4.131	0.000	-0.070	-0.025
5	-0.363	0.014	-4.960	-1.291	0.009	0.026	0.170
6	0.385	0.196	-4.326	-2.313	0.001	0.069	-0.180
7	0.107	-0.014	7.426	7.999	0.018	0.066	0.055
8	-0.572	-0.027	2.372	2.687	0.007	-0.067	0.048
9	-0.039	0.373	3.072	5.062	0.015	0.009	-0.167
10	-0.068	0.236	-2.366	-8.765	-0.042	0.023	0.053
LSD(0.05)	0.035	0.137	3.596	2.843	0.033	0.015	0.056

Table 7. Summary heritability estimates for optimum and drought environments.

Trait	Optimum	Drought	Optimum	Drought
Grain yield (t/ha)	7.97±0.62	1.74±0.57	49.3	38.7
Anthesis silking interval (days)	0.64±0.30	2.14±0.49	33.5	40
Ears per plant	1.15±0.10	0.95±0.11	37.2	53.6
Senescence (1-10)	3.61±0.41	5.58±0.72	39.6	51.4
Plant height (cm)	230±8.52	202±7.31	64.5	52.3
Exserhileum turcicum (score 1-5)	2.76±0.39	N/A	84.6	N/A

positive correlations with both optimum (Y_p) and stress (Y_s) environments. ASI had strong and negative correlation with Y_s , and a strong positive significant correlation with YSI. EPP had significant correlation with Y_s , Y_p and YSI but had strong negative correlation with ASI (Table 8).

DISCUSSION

Genotype x environment (GxE) effects

The significant interaction of line x tester (SCA) with the environment showed that genotypes were performing differently in different environments thereby allowing selection differentials for GY and ASI since some hybrids were distinctly superior. SCA effects for GY, ASI and EPP were highly significant ($P < 0.001$), under drought an indication that the secondary traits EPP and ASI are good proxies for GY under stress environments. The G x E effects of ASI and EPP under optimum conditions were not significant but differentials were observed under moisture stress. Both line and tester GE effects were significant showing that ASI is an even more reliable trait for selection of synchronization and consequently yield under moisture stress. The heritability values of the two traits (EPP and ASI) increased with stress making it possible to separate the hybrids for good performance

under the moisture stress environment. This therefore means that the two traits can be used to select for stress tolerance. Similar findings were reported by Banziger et al. (2001) and Ribaut et al. (2004) where breeding value of the two secondary traits increased with increase in moisture stress. The L10 x T5 SCA for GY combination result also confirms previous findings that good specific combiners are not necessarily good general combiners and vice versa. This is further confirmed by the results where L2 and T3 (Table 6) were good general combiners, but had a negative SCA of $-0.572t$ (Table 5). Generally negative SCA effects are a result of crosses involving inbred lines with the same genetic background, while being positive for crosses involving inbred lines with a divergent genetic background (Vasal et al., 1992; Betran et al., 2003).

Indices and correlations

There seems to be an inverse relationship between STI and TOL where the more STI the less the TOL. The general trend also showed an increase in SSI from the best to the worst performer while YSI and EPP values reduced with an increase in SSI. The trend is the same when one looks at the susceptible check 1 (SC727) and the tolerant check 2 (CLM539/442). Check 1 was the best yielding variety under optimum conditions but its yield

Table 8. Correlation coefficients between Yp, Ys and drought tolerance indices.

Variable	Yp	Ys	SSI	STI	TOL	GMP	MP	YI	YSI	ASI	EPP	SEN
Yp	1.000											
Ys	0.298*	1.000										
SSI	0.618**	0.433**	1.000									
STI	0.732**	0.848**	0.001	1.000								
TOL	0.849**	0.251	0.866**	0.273*	1.000							
GMP	0.762**	0.841**	0.072	0.985**	0.308*	1.000						
MP	0.913**	0.662**	0.299*	0.938**	0.560**	0.958**	1.000					
YI	0.296*	1.000**	0.435**	0.847**	0.253	0.839	0.660**	1.000				
YSI	0.620**	0.431**	1.000**	0.002	0.866**	0.074	0.302*	0.433**	1.000			
ASI	0.008	0.379**	0.527**	0.144	0.379**	0.230	0.300*	0.006	0.528**	1.000		
EPP	0.356**	0.283**	0.472**	0.273*	0.260	0.36**	0.357**	0.182	0.471**	0.405**	1.000	
SEN	0.227	0.117	0.141	0.179	0.165	0.205	0.229	0.116	0.135	0.077	0.161	1.000

*P<0.05; **P<0.01; Red or italicised values are negative values.

was significantly reduced under drought conditions as shown by a TOL index value of 8.9t. This shows that the two indices can be reliably used in assessing a variety for grain yield across optimum and stress conditions. The STI values can also show that stable varieties across the 2 water regimes are not necessarily the highest yielding. Correlation between Ys and Yp was positive and significant ($r = 0.298^*$) implying that there were significant differential among genotypes to enable effective selection for drought tolerance. This is in tandem with findings by Banziger et al. (2000). A general linear model of GY under drought stress on STI showed a coefficient of determination ($R^2 = 0.72$) which is quite high in helping explain cultigen performance. In this study GMP was used because it's more reliable and robust since it is relative to cultigen performance across environments and seasons. EPP also had significant ($P<0.01$) and positive correlation with Ys and Yp, STI ($P<0.05$), GMP ($P<0.01$) showing that this trait is reliable and can be used in determining cultigen performance across both stress and non stress environments.

This is similar to findings by Khayatnezhad et al. (2010) working on drought stress in wheat. ASI had significant and negative correlation with Ys and TOL ($r = -0.379^{**}$) showing that this trait is also reliable for use under stress environments as low values of ASI are ideal when selecting genotypes for stress tolerance. The ASI –TOL negative relationship also further confirms that low value ASI genotypes have low yield reduction under stress environments and vice versa and hence the two can be used in selection for genotypes that perform across environments. ASI and EPP had significant negative correlation ($P<0.01$). These are inversely proportional given that genotypes with high EPP value have a low ASI values and vice versa. The significantly negative correlation ($r = 0.433^{**}$) for SSI under drought conditions shows that plant environment has a decisive factor in yield. This is also confirmed by Mitu (2003), in his

research on drought and heat tolerance in maize genotypes. This further demonstrates that evaluation and reliability of the indices and the secondary traits used depends on the level or severity of stress under which the genotypes are exposed. YSI and SSI have a negative perfect correlation and can therefore be used interchangeably depending on the direction of selection a breeder might want to follow.

Gene action and breeding value

The GCA was more predominant over SCA in grain yield, anthesis-silking interval, ears per plant and senescence under drought conditions compared to the optimum environments. This means that the breeding value or repeatability increased in the drought stress conditions as shown in Table 8. Despite the small value increase in heritability for the traits under study the general trend is similar to findings by Betran et al. (2003) who reported 24% increase in additive genetic variance under drought over well watered environments. These results therefore suggest the need for use of all drought tolerant parents in hybrid combinations if there are to be significant yield gains to be obtained under drought stress environments. However no significant differences were observed for disease under drought to warrant data reporting. Among the agronomic traits measured plant height and grain yield were the only traits with reduced heritability under stress. This can be explained by the fact that maize above ground biomass is strongly correlated to grain yield. This is also because estimated QTL effects for traits such as grain yield or plant height have limited transferability in stress environments. The results found are consistent with most research work done in stress environments where biomass is reduced due to reduced increased prioritization of assimilates to grain formation rather than apical dominance (Banziger et al., 2001;

Betran et al., 2003). In breeding and selecting for drought tolerance one would therefore favour the secondary trait of ASI where there is minimal flowering asynchrony between male and female flowering structures (that is, a short ASI) and high EPP or reduced bareness.

Conclusions

GCA effects had predominance over SCA effects for grain yield, ASI and EPP under stress. This therefore confirmed that the secondary traits ASI and EPP can be used as proxies for GY under stress environments. In this study phenotypic variation explained by additive genetic effects increased with an increase in severity of stress. The secondary traits EPP and ASI were shown to be effective in selection for both stress and non stress environments. STI and GMP were the most reliable among the selection indices used in this study and therefore we recommend that they be used in selection. We further recommend that a combination of factors have to be considered for the breeder to make an informed decision when selecting ideal genotypes. This will include knowledge of parental lines in terms of gene action and breeding value of traits as well as using secondary traits and drought indices. Therefore, secondary traits and selection indices should be used in tandem for one to make an informed decision on selecting the best and most ideal genotypes. However the severity of the stress (selection intensity) will also determine the level of contribution of the different traits and the usefulness of a given index.

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