

**Identification of drought, heat, and combined drought and heat tolerant donors in maize (*Zea mays* L.)**

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**ABSTRACT**

Low maize yields and the impacts of climate change on maize production highlight the need to improve yields in eastern and southern Africa. Climate projections suggest higher temperatures within drought-prone areas. Research in model species suggests that tolerance to combined drought and heat stress is genetically distinct from tolerance to either stress alone, but this has not been confirmed in maize. In this study we evaluated 300 maize inbred lines testcrossed to CML539. Experiments were conducted under optimal conditions, reproductive stage drought stress, heat stress and combined drought and heat stress. Lines with high levels of tolerance to drought and combined drought and heat stress were identified. Significant genotype x trial interaction and very large plot residuals were observed; consequently, the repeatability of individual managed stress trials was low. Tolerance to combined drought and heat stress in maize was genetically distinct from tolerance to individual stresses, and tolerance to either stress alone did not confer tolerance to combined drought and heat stress. This finding has major implications for maize drought breeding. Many current drought donors and key inbreds used in widely-grown African hybrids were susceptible to drought stress at elevated temperatures. Several donors tolerant to drought and combined drought and heat stress,

notably La Posta Sequia C7-F64-2-6-2-2 and DTPYC9-F46-1-2-1-2, need to be incorporated into maize breeding pipelines.

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Abbreviations: ASI, anthesis-silking interval; CIMMYT, International Maize and Wheat Improvement Center; ESA, eastern and southern Africa; *H*, broad sense heritability, IITA, International Institute of Tropical Agriculture, PH, plant height; SSA, sub-Saharan Africa; VPD, vapour pressure deficit.

## INTRODUCTION

In eastern and southern Africa (ESA), maize is the most important crop, accounting for up to 40 to 50% of both calories and protein consumed in Malawi, Zimbabwe, and Zambia, the most maize-dependent countries in the region (FAOSTAT, 2010). However, maize yields in this region remain low averaging  $1.4 \text{ t ha}^{-1}$ , or one-sixth the average yields in the USA (FAOStat, 2010). These yield levels are barely enough to ensure food security, and often fall short. The need to increase maize yields for food security in ESA is heightened by both population growth and climate change. The population of sub-Saharan Africa is predicted to double by 2045 (World Population Prospects, median variant; United Nations, 2009), while climate projections for ESA show decreasing precipitation, increasing temperatures, and a higher frequency of extreme events (IPCC, 2007). A comparison of different cropping systems across regions identified maize production in southern Africa as one of three climate risk hotspots (Lobell et al. 2008). Drought has long been recognised as a major constraint to maize yields in this region (Heisey and Edmeades, 1999), however, heat stress both alone and in combination with drought stress is likely to become an increasing constraint to maize production (Cairns et al. 2012a). Lobell and Burke (2010) showed that an increase in temperature of  $2 \text{ }^{\circ}\text{C}$  would result in a greater reduction in maize yields than a decrease in precipitation of 20 %. Similarly, a recent study in Tanzania also indicated that increasing temperatures would result in a greater reduction in maize yields than increased intra-seasonal variability in precipitation (Rowhani et al. 2011). In this study a projected increase in temperature of  $2 \text{ }^{\circ}\text{C}$  reduced maize yields by 13 %, while a 20 % increase in intra-seasonal variability reduced maize yields by only 4.2 %. These studies

highlight the need to incorporate heat tolerance, as well as increased drought tolerance, into African maize germplasm to offset predicted yield losses.

Substantial progress has been made in drought breeding in subtropical and tropical maize. In the 1970s CIMMYT initiated a drought breeding programme for maize using the elite lowland tropical maize population “Tuxpeño Sequia” (Bolaños and Edmeades, 1993a,b; Bolaños et al., 1993). Over eight cycles of full-sib recurrent selection for grain yield and increased flowering synchronisation (reduced anthesis-silking interval (ASI)) resulted in gains of up to 144 kg ha<sup>-1</sup> yr<sup>-1</sup> under drought stress (Edmeades et al. 1999). In the late 1990’s CIMMYT initiated a product-orientated maize breeding programme in southern Africa (Bänziger et al. 2006). Maize varieties were simultaneously selected for performance under optimal, low nitrogen and managed drought stress conditions. CIMMYT hybrids yielded more than commercial checks at all yield levels. Under severe stress CIMMYT hybrids had a 40 % yield advantage compared to commercially available hybrids. Recent on-farm trials in ESA of new hybrids showed a 35 % and 25 % yield advantage against farmers own varieties under low (< 3 t ha<sup>-1</sup>) and high yield conditions, respectively (Setimela et al. 2012). The best hybrid (CZH0616) out-yielded the most popular commercial check, which was released approximately 15 years ago, by 36 % and 26 % under high and low yield conditions, respectively, indicating that gains from selection for both yield potential and stress tolerance have been large.

In contrast to drought research, relatively less effort has been devoted to breeding specifically for heat stress tolerance in maize. Earlier studies highlighted the negative impact of

increased growing season temperatures on temperate maize yields. Thomson (1966) showed that an increase in temperature from 22 °C to 28 °C during the grain filling period in the US Corn Belt resulted in a 10 % yield loss, while Badu-Apraku et al. (1983) showed a 42 % yield reduction when mean daily temperatures were increased by 6 °C. A recent analysis of more than 20,000 historical maize trial yields in southern Africa showed that maize production linearly decreased with every accumulated degree day above 30 °C (Lobell *et al.* 2011). Heat stress in maize is associated with shortened life cycle (Muchow et al. 1990), reduced light interception (Stone, 2001), increased respiration, reduced photosynthesis (Crafts-Brander and Salvucci, 2002) and pollen sterility (Schoper et al. 1987a, b). A comparison of the response of male and female reproductive tissues to heat stress demonstrated that female tissues have greater tolerance (Dupis and Durnas, 1990), with pollen production and/or viability highlighted as major factors controlling reduced fertilisation under high temperatures. However the period between silk pollination and ovary fertilisation in the female reproductive tissues has also recently been highlighted as a critical period controlling grain yield under heat stress (Cicchino et al. 2011). Given the diversity of ecosystems in which maize can be grown, it is highly likely that there is genetic variability in the tolerance of tropical and sub-tropical maize to heat stress. However, tropical maize has been shown to have stable yields across a narrower range of temperatures than temperate maize (Lafitte et al. 1997). Climate predictions show an increase in growing season temperatures within drought prone regions (Cairns et al. 2012a). While drought stress is often a combination of water and temperature stress as a result of reduced transpirational cooling under limited water conditions, there is evidence to suggest that the response to drought stress at elevated ambient temperatures is unique and cannot be extrapolated from

the sum of the effects of both stresses (drought and heat) (Rizhsky et al. 2002; 2004; Barnabás et al. 2008).

Both conventional and molecular breeding approaches rely on genetic variability for the trait of interest. The aim of this study was therefore to identify lines with tolerance to drought, heat and combined heat and drought stress for use within maize breeding programs, to serve both as donors of drought and heat tolerance in pedigree breeding and as potential sources of alleles with large effects detectable through genome-wide association mapping (to be reported in a subsequent paper). We evaluated a diverse set of 300 inbred lines from CIMMYT and IITA's tropical and subtropical breeding programmes as testcrosses to the broadly-adapted line CML539 in drought- and heat-stressed field trials in Kenya, Zimbabwe, Mexico, and Thailand. Additional aims of this study were to establish the relationship between drought, heat and combined drought and heat tolerance in maize, and to assess the magnitude of genotype x trial plot residual variances in managed drought and heat stress trials with respect to their effects on the repeatability of field phenotyping for these stresses.

## **MATERIALS AND METHODS**

### *Plant material*

A collection of 300 inbred lines was assembled, representing the genetic diversity within the CIMMYT and IITA tropical and subtropical maize improvement programmes (Wen et al. 2011). Briefly, lines were assembled from nine CIMMYT and IITA breeding programmes based in Latin America and Africa focussing on yield potential and abiotic and biotic stress tolerance (Table 1).

Information on the pedigree and adaptation zones of all lines is presented in Table S1. Single cross hybrids were generated by crossing lines with the tropical tester CML-539, a broadly-adapted inbred that is tolerant to maize streak virus, a disease prevalent only in Africa but not elsewhere.

### *Trial management*

Trials were conducted at CIMMYT maize experimental stations in Tlaltizapán, México (18°41'N, 99°07'W, 940 masl), Kiboko, Kenya (2°21'S, 37°72'E, 975 masl) and Chiredzi, Zimbabwe (21°01'S, 31°34'E, 430 masl), at the Nakhonsawan Field Crops Research Center in Takfa, Thailand (15°21'N, 100°30'E, 87 masl), and at the ICRISAT experimental station in Hyderabad, India (7°53'N, 78°27'E, 545 masl). The soil of the experimental field in Mexico is a clay loam with a pH of 7.6 and classified as an Isothermic Udic Pellustert. In Kenya the soil is sandy clay with a pH of 7.9 and classified as an Acro-Rhodic Ferrasol. In Zimbabwe the soil is a clay loam with a pH of 5.5 and classified as a Typic Rhodustalf. In Thailand the soil is a clay loam. In India the soil is 50 % clay with a pH of 8.5 and classified as a Typic Pellustert.

In the experiment conducted in Mexico in 2009, hybrids were separated into four maturity groups; early (50 entries), early-intermediate (100 entries), intermediate late (100 entries) and late (50 entries). In 2010 and 2011 phenology groups were redefined into two maturity groups, early (150 entries) and late (150 entries) in all locations. Experiments were planted during the dry season in all locations with the exception of India to allow drought stress to be imposed at the anthesis stage (Table 2). Experiments were planted either in two-row plots

(Mexico and Thailand) or one-row plots (Kenya and Zimbabwe), with a final plant density of 6.67 plants m<sup>-2</sup> (Mexico, Kenya, India and Zimbabwe) or 5.33 plants m<sup>-2</sup> (Thailand). At all locations two seeds per hill were sown, then thinned to one after emergence. An alpha-lattice design was used, replicated three times in 2009 and two times in 2010 and 2011. All plots received 80 kg N ha<sup>-1</sup> (as urea), 80 kg P ha<sup>-1</sup> (as triple calcium superphosphate; Ca (H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>H<sub>2</sub>O) at sowing. A second application of N (80 kg N ha<sup>-1</sup>) was applied 5 weeks after sowing (V6 stage, Ritchie et al., 1993). Recommended plant, weed, and insect control measures were used.

Two different water regimes were used; a well-watered control and anthesis stage drought stress. Drought stress was imposed by stopping irrigation before flowering to ensure stress at anthesis. Rainfall, temperature, and vapour pressure deficit (VPD) data during experiments are presented in Figure 3. The weather station in Mexico in 2011 malfunctioned for 39 days between the period 18<sup>th</sup> March to 5<sup>th</sup> June; no weather data is available on these days. In Zimbabwe relative humidity was not measured so it was not possible to calculate VPD. In Kenya in 2010 the early maturity group did not experience drought stress at anthesis due to unexpected rain at this stage and this subset was excluded from the analysis. Delayed planting in the dry season in both Mexico and Zimbabwe allowed high temperatures during the reproductive stage. In Mexico in 2010, daily maximum temperatures exceeded 35 °C during the reproductive phase for 8 and 52 days in the drought and combined drought and heat trials, respectively. In Zimbabwe in 2010, daily maximum temperatures exceeded 35 °C during the reproductive phase for 37 days in the combined drought and heat trial. In India in 2011, daily maximum temperatures exceeded 35 °C during the reproductive phase for 31 days (out of the



55 days from anthesis to physiological maturity) in the heat trial. In Kenya in both 2010 and 2011, maximum temperatures never reached 35 °C during the reproductive stage. In Thailand daily maximum temperatures exceeded 35 °C during the reproductive phase for 35 and 22 days in the drought trials in 2010 and 2011, respectively. However, VPD was very low in Thailand compared to the combined drought and heat stress trials in Mexico and heat trials in India.

Days to anthesis and silking were recorded when 50 % of the plants had shed pollen and 50 % of the plants had silks, respectively. The ASI was calculated as days to silking – days to anthesis. At physiological maturity, plant height was measured on two representative plants per plot, then all plants were hand harvested and grain yield measured. Grain weights were adjusted to 12.5 % moisture content.

### *Statistical analysis*

Variance components were estimated by restricted maximum likelihood (REML) using Proc Mixed of SAS v9.0. The standard linear mixed model for the response variable is represented by

$$y_{ijkb} = \mu + E_j + R(E)_{kj} + IB(RE)_{bkj} + G_i + GE_{ij} + e_{ijkb}$$

where  $\mu$  is the overall mean,  $E_j$  is the effect of the  $j^{\text{th}}$  location,  $R(E)_{kj}$  is the effect of the  $k^{\text{th}}$  replicate within the  $j^{\text{th}}$  location,  $IB(RE)_{bkj}$  is the effect of the incomplete block within the  $k^{\text{th}}$  replicate in the  $j^{\text{th}}$  environment,  $G_i$  is the effect of the  $i^{\text{th}}$  genotype, and  $GE_{ij}$  is the interaction effect of the  $i^{\text{th}}$  genotype with the  $j^{\text{th}}$  location. All effects, except locations, were considered

random and Best Linear Unbiased Predictors (BLUPs) were computed for all tropical and sub-tropical maize testcrosses. The SAS code is presented in supplemental data 2.

Broad-sense heritability ( $H$ ) was estimated as  $H = \sigma_g^2 / (\sigma_g^2 + \sigma_{g \times e}^2 / e + \sigma_e^2 / re)$  where  $\sigma_g^2$  is the genotypic variance,  $\sigma_{g \times e}^2$  is the genotype x environment and  $\sigma_e^2$  is the residual variance.  $e$  is the number of environments, and  $r$  is the number of replicates per environment.

The genetic correlations between environments/season ( $i^{\text{th}}$  and  $j^{\text{th}}$ ) are calculated using equations from Cooper et al. (2006) by

$$\rho_g = \overline{\sigma_{g(ii)}} / \overline{\sigma_{g(i)} \sigma_{g(i')}}^2$$

where  $\overline{\sigma_{g(ii)}}$  is the arithmetic average of all pairwise genotypic covariances between environments  $i^{\text{th}}$  and  $i'^{\text{th}}$ , and  $\overline{\sigma_{g(i)} \sigma_{g(i')}}^2$  is the arithmetic average of all pairwise geometric means among the genotypic variance components of the environments

## RESULTS

### *Grain yield, phenology and plant height across different environments*

Under non-stress conditions, average trial grain yields ranged from 6.50 to 7.39 t ha<sup>-1</sup> (Table 2). Drought stress significantly reduced grain yield in all trials, with the reduction in yield ranging from 55 % to 75 % of the well-watered control. Mean grain yield under drought stress ranged from 1.70 t ha<sup>-1</sup> (Mexico 2009) to 3.30 t ha<sup>-1</sup> (Mexico 2011). In Mexico and Kenya large variation was observed in mean grain yield under drought stress across years. Mean grain yield under combined drought and heat stress ranged from 0.24 t ha<sup>-1</sup> (Mexico 2010) to 2.25 t ha<sup>-1</sup> (Mexico

2011). Grain yield under heat stress was 1.50 t ha<sup>-1</sup> in India. ASI ranged from 1.79 (Kenya 2010) to 5.11 (Thailand 2010) under drought stress, 2.64 (Mexico 2011) to 12.01 (Mexico 2010) under combined heat and drought stress, and 0.58 (Thailand 2010 and 2011) to 2.40 (Mexico 2010) under well-watered conditions. Combined drought and heat stress in Mexico significantly reduced the average number of days to anthesis relative to drought trials by 15.5 days in 2010 and 13.4 days in 2011. Similarly, plant height was reduced by 38.2 cm and 19.2 cm in the combined drought and heat stress trials relative to drought trials in 2010 and 2011, respectively.

*Variance components and broad-sense heritability under drought, heat, combined drought and heat stress and optimal conditions*

$H$  estimates for grain yield in individual trials ranged from 0.64 to 0.84 under well-watered conditions, 0.64 to 0.82 under heat stress, 0.47 to 0.80 under drought stress and 0.32 to 0.72 under combined drought and heat stress (Table 2). In the combined analysis, the  $H$  of grain yield was lower under stress relative to non-stress (Table 3), however  $H$  was above 0.50 in all treatments. In drought trials ( $n = 7$ ),  $H$  was 0.64 while under combined drought and heat stress ( $n = 3$ )  $H$  was 0.50. Under drought stress and combined drought and heat stress the  $\sigma_e^2$  and  $\sigma_{g \times e}^2$  variances were much higher relative to  $\sigma_g^2$  than in well-watered trials. In all well-watered trials (with the exception of the well-watered trial in Mexico in 2011)  $\sigma_g^2$  accounted for the largest proportion of the phenotypic variance. In contrast  $\sigma_e^2$  generally accounted for the largest proportion of the phenotypic variance in drought and combined drought and heat stress trials.

In the combined analysis across trials within stress levels, the pooled plot residual variance was similar in magnitude to the genotypic variance under well-watered conditions, but was two to three times larger under stress conditions.

#### *Correlations between treatments, trials and phenology*

Grain yield under well-watered conditions was moderately positively genetically correlated with grain yield under drought stress (Table 4), with approximately 40% of genetic variance for yield under drought stress alone accounted for by variation in yield potential under well-watered conditions. Grain yield under combined drought and heat stress was weakly positively correlated with grain yield under well-watered conditions, but genetic variation in yield potential explained only about 5% of variability under combined drought and heat stress. There was no significant relationship between grain yield under drought stress and combined heat and drought stress. Grain yield was positively correlated with days to anthesis under well-watered conditions ( $r = 0.51$ ,  $p < 0.001$ ), however, there was no significant relationship between grain yield under drought stress and days to anthesis ( $r = 0.03$ , ns). Under combined drought and heat stress, grain yield was weakly negatively associated with days to anthesis ( $r = -0.3$ ,  $p < 0.01$ ), while for heat stress grain yield was weakly positively correlated with days to anthesis ( $r = 0.25$ ,  $p < 0.01$ ).

Genetic correlations for grain yield between individual trials are presented in Table 5. In general, within individual locations, genetic correlations for grain yield for the same treatment were strongly, positively correlated between years, except for drought trials in Mexico in 2009

and 2010 ( $r = 0.02$ , ns) and Kenya in 2010 and 2011 ( $r = -0.01$ , ns), however in Kenya only the late maturity group in 2010 were included in the analysis due to rainfall at anthesis in the early maturity group. In both Mexico and Thailand, well-watered trials were strongly, positively correlated with the respective drought trials in the same year. In Mexico, well-watered trials were weakly and positively correlated with the respective combined drought and heat stress trials in the same year. Heat trials in India were positively correlated with all drought and well-watered trials in Mexico, Thailand and Kenya. There was no significant correlation between grain yield under heat stress in India and grain yield under combined heat and drought stress trials in Mexico. In Mexico under combined drought and heat stress yields were higher in 2011 relative to 2010 and there was no significant difference in grain yield between drought and combined heat and drought stress trials in 2011. However there was still a weak yet significant negative correlation between the two treatments.

## ***DISCUSSION***

### *Comparison of drought, heat and combined drought and heat stress*

The combination of water and temperature regimes across trials allowed the relationship between individual and combined stresses to be established. Yield losses were higher under drought stress when temperatures were elevated as recently reported by Lobell et al. (2011). In agreement with previous studies on abiotic stress, grain yield under both drought stress and heat stress was moderately and positively associated with grain yield under well-watered conditions (Bänziger et al. 1997), but correlations are not high enough for performance under well-watered conditions to be predictive under drought stress or heat stress; approximately 60

% and 85 % of the genetic variance for yield under drought and heat stress, respectively, was unique to each environment relative to well-watered conditions. Grain yield under optimum (well-watered) and combined drought and heat stress had a weak positive correlation indicating the presence of independent genetic control of yield in the two environments. Performance under drought stress was moderately correlated with performance under heat stress. The moderate relationship between tolerance to drought and heat stress has previously been reported in both wheat and rice (Jagadish et al. 2010; Pinto et al. 2011). However, grain yield under drought stress or heat stress was not associated with grain yield under combined drought and heat stress. Although the effect of plant drought stress is often a combination of the effects of drought and heat through reduced transpirational cooling, these results are in agreement with previous studies where drought stress was genetically distinct from drought stress under elevated temperatures.

The majority of research on abiotic stresses has focussed on individual stresses, while in farmers' fields plants are regularly subjected to a combination of stresses (Voisenik et al., 2008). Independent screening for tolerance to drought and low nitrogen in tropical maize has resulted in germplasm with tolerance to both stresses (Bänziger et al. 2000; 2006). However, these results show that tolerance to combined drought and heat stress in maize is genetically distinct from tolerance to the individual stresses and screening under drought or heat alone will not confer tolerance to the combined effect of drought and heat stress. Even in trials in Mexico in 2011 when there was no significant difference in mean trial yields under drought and combined drought and heat stress, there was no relationship between the two treatments,

with considerable lack of agreement in the ranking of genotypes for grain yield between the two treatments.

Increasing temperatures are highly likely to result in large yield losses in maize production in SSA (Jones and Thornton, 2003; Lobell et al. 2008; Rowhani et al. 2011). Compared to other abiotic stresses associated with climate change (drought stress and water-logging), relatively little research has been conducted on heat stress in maize. The vast majority of studies have focussed on biochemical and molecular responses using only a limited number of genotypes with stress applied *in vitro* as a single and rapid heat stress event, rather than in response to heat under field conditions (Cairns et al. 2012b). The current study has shown large genetic variation in grain yield under heat stress in sub-tropical and tropical maize germplasm in the field. To date there has been no extensive breeding effort that targets specifically heat stress in tropical and sub-tropical maize. Several potential donors with tolerance to heat stress were identified in this study, however further trials are needed to confirm these results. Combined drought and heat stress is likely to become an increasing constraint to maize production in SSA, particularly in the drought-prone lowlands of southern Africa (Cairns et al. 2012b). Our results indicate that current maize germplasm developed for drought tolerance may not perform well under drought stress at elevated temperatures. Thus maize breeding for tolerance to drought stress under elevated temperatures must include screening under the combined effect of drought and heat rather than the individual stresses.

*Key donors for drought and combined drought and heat stress*

The screening of a large number of advanced lines from many breeding programs across multiple locations has clearly identified highly drought tolerant lines. The ten testcrosses with the highest grain yield under drought stress and combined drought and heat stress are presented in Tables 6 and 7, respectively (grain yield BLUPs for all testcrosses in each environment are also presented in Table S1). These testcrosses yielded at least  $0.65 \text{ t ha}^{-1}$  more under drought stress than the current donor used in drought breeding (CML444) crossed to the broadly-adapted tester CML539. The subtropical line [SYN-USAB2/SYN-ELIB2]-12-1-1-2 developed in Zimbabwe for mid-altitude environments had the highest yield under drought stress, with  $0.81 \text{ t ha}^{-1}$  more than the current drought tolerant line (CML444) crossed to CML539. However this line (crossed to CML539) yielded less than the trial means under both combined heat and drought stress and heat stress alone. Indeed [SYN-USAB2/SYN-ELIB2]-12-1-1-2 was one of the most susceptible genotypes to combined drought and heat stress. Similarly, both DTPWC9-F2-3-2-1 (a tropical line derived from a recurrent selection programme for drought tolerance) and CLQ-RCYQ40 = (CML165 x CLQ-6203)-B-9-1-1 (a tropical line developed in Mexico) crossed to CML539, were highly tolerant to drought stress yet ranked within the bottom third of lines under combined heat and drought stress. In contrast, La Posta Seq C7-F64-2-6-2-2 and DTPYC9-F46-1-2-1-2, developed for drought tolerance through recurrent selection in Mexico, were high yielding relative to the trial mean under both drought and combined drought and heat stress. These lines were selected under a range of environments including one heat screen (Edmeades et al. 1999). The drought tolerant hybrid, CML442/CML444, was found to be highly susceptible to drought stress under moderately elevated temperatures. CML442/CML444 has been used extensively as the female parent in hybrid development in



eastern and southern Africa (MacRobert, *pers. comm.*) and to ensure that drought tolerant commercial maize varieties continue to perform under future climates, a new female parent must be developed with tolerance to drought stress at higher temperatures.

The size of this study (ca. 18,600 rows) prevented the use of more than one tester from different heterotic groups. CML539 was chosen as the tester because it has good combining ability across heterotic groups. In general CIMMYT germplasm is extremely diverse and does not separate neatly into two or even more genetic groups; there are at least 8 distinct subpopulations, in terms of breeding origin, with the set (Wen et al. 2011). However the risk of heterotic or genetic group differences being confounded with the main effects observed is low. Furthermore, many of the donors identified in this study have been confirmed independently in other unpublished experiments.

Steady gains in maize breeding for tolerance to drought stress have been made (Bänziger et al. 2006; Setimela et al. 2012). If the donors identified in this study are incorporated into drought breeding pipelines they will further improve gains under drought stress. Six of the top 10 DT donors were from drought tolerant populations (La Posta Sequia and DTP) developed in the 70's, 80's, and 90's through recurrent selection (Bolaños and Edmeades, 1990; Edmeades et al. 1997a). Subsequent research at CIMMYT has focussed on product development (Edmeades et al. 1997b). Little additional gain in maximum drought tolerance has been achieved, as evidenced by the fact that the most tolerant lines in this panel were derived from populations developed and improved under the CIMMYT Physiology program over 15

years ago. These results highlight the need for the identification of new drought tolerant source populations, and for continued development of donors with higher levels of tolerance. Only a small proportion of the genetic variation within maize has been exploited (Ortiz et al. 2009). Landraces from areas that frequently have high temperature may provide a useful source of novel alleles for heat tolerance (Castro-Nava et al. 2011). While the development of new source populations through conventional methods is slow, genomic selection may provide a faster alternative through rapid-cycle, marker-based recurrent selection (Weber et al. 2012a)

#### *Implications for maize breeding in SSA*

Breeding progress relies on genetic variability for the trait of interest, high selection intensity, high  $H$  for the trait of interest and the genetic correlation between yield in the selection environment and the TPE (Falconer, 1952).  $H$  values of single trials under stress were moderately high but consistent with published studies of maize trials under managed drought stress (Bolaños and Edmeades, 1996; Messmer et al. 2009; Almeida et al. 2012; Weber et al. 2012). Selection for increased flowering synchrony (e.g. reduced ASI) has been successfully used in maize breeding for drought prone environments (Bänziger et al. 2006). Although  $H$  was generally higher for GY than ASI, this is in agreement with previous studies (Bolaños and Edmeades, 1996; Messmer et al. 2009; Almeida et al. 2012) and selection efficiency for grain yield under drought stress increased when reduced ASI was used in combination with grain yield (Bänziger and Lafitte, 1997). However  $H$  in single trials inflated because genetic variance is confounded by genotype x trial interactions. In the combined analysis under optimal conditions,  $\sigma_g^2$  was comparable to  $\sigma_{g \times e}^2$ , however, under both drought stress and combined drought and

heat stress,  $\sigma_{g \times e}^2$  was two-fold to three-fold greater than  $\sigma_g^2$  (Table 3). Similarly,  $\sigma_e^2$  was two to three-fold greater than  $\sigma_{\text{genotype}}^2$  in stress trials. The very large residual and genotype x trial variances in the drought and combined drought and heat treatments relative to the well-watered treatment resulted in reduced  $H$  for means estimates from the stressed trials. A recent combined analysis of the southern Africa regional trials of CIMMYT and partners also found plot residual variance to be much higher under managed stress relative to non-stress trials (Weber et al. 2012b). Although the  $H$  of managed stress trials in this study was lower than that of non-stress trials and plot residual variance under abiotic stress was high,  $H$  was above 0.5 for a treatments and the repeatability of trials across years provided a good estimate of the performance of lines for breeding purposes. However these results highlight the need for measures to reduce the effects of field variability so as to increase the genetic signal to noise ratio to detect real differences between lines. Reducing the size of the residual relative to the genetic component of variance would have a positive impact on heritability levels and expected genetic gains. Soil heterogeneity can represent a significant source of experimental variation and obstruct the detection of the genetic signal (Campos et al. 2011). Although the sites used in this study were previously selected for relative uniformity, soil is inherently heterogeneous. Measures of soil variability can be incorporated into statistical analysis to reduce experimental error (Hao et al. 2010, Cairns et al. 2011; Cairns et al. 2012b). However, the value of these measures still needs be established in reducing the error variance relative to the genetic variance (Masuka et al. 2012). Genotype x trial interaction was also much greater in drought and combined drought and heat trials relative to the genotypic variance (Table 3). It is important to recognise that the genotype x trial interaction is a true error stratum in managed

stress screening, and an important source of noise reducing repeatability and increasing standard errors of means. Because of this, it is critical that germplasm evaluation under managed stress be replicated in several trials to sample these fluctuations and ensure that measures of precision of the estimates of means are realistic. These results also highlight the need for measures to reduce trial-to-trial variability that are likely to be associated with the timing and severity of drought stress in germplasm screening. Similar irrigation regimes were applied at each site to ensure drought stress at the flowering stage, but variation in rainfall and temperature across years is likely to have resulted in different drought stress profiles. Soil moisture monitoring has routinely been used in rice for drought breeding, to reduce variation in drought stress between experiments and years (Lafitte et al. 2006; Venuprasad et al. 2007 and 2008) and needs to be applied within maize breeding for drought tolerance in ESA.

## **CONCLUSIONS**

A systematic evaluation of elite material derived from breeding programmes worldwide under a range of environments allowed the identification of donors with high levels of tolerance to both drought and combined drought and heat stress. Incorporating these donors into drought breeding pipelines will help increase genetic gains. However, little additional improvement in maximum drought tolerance has been achieved since the 1990s. These results highlight the need to develop new drought tolerance source populations for maize improvement strategies that target drought-prone areas. Maize plants in farmers' fields are routinely subjected to a combination of stresses. The occurrence of drought and heat stress together is likely to increase, particularly in southern Africa. The results of this study suggest that the genetic

control of drought, heat, and combined drought and heat tolerance are largely independent of each other. Current drought tolerant parents used in hybrid maize breeding in ESA are highly susceptible to drought stress under elevated temperatures and there is a need to incorporate lines with tolerance to combined drought and heat stress in drought-prone areas where temperatures are predicted to increase. Only a limited relationship was found between yield potential and stress tolerance, with less than 40% of genetic variance in tolerance explained by variation in yield potential in all cases. This confirmed that performance under well-watered conditions is not predictive enough of performance under both drought and heat stress. The independent genetic control may indicate that QTLs with large effects are involved in combined drought and heat and heat tolerance.

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Figure 1. Weather records a) Mexico 2009, b) Mexico 2010, c) Mexico 2011, d) Thailand 2010, e) Thailand 2011, f) Kenya 2010, g) Kenya 2011, h) Zimbabwe 2010 and i) India 2011. Daily average air temperature (hollow circles), maximum air temperature (crosses), VPD (solid line) and rainfall (columns) are presented for the growing season at each location.

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Table 1. Origin of germplasm used in this study.

Location/group	Main target of programme	Number of lines	Grain colour	
			White	Yellow
Colombia	Soil acidity	27	4	23
Entomology	Pest resistance	48	33	15
Highland	Yield potential	5	1	4
Physiology	Drought and low nitrogen	84	65	19
Sub-tropical	Yield potential	40	25	16
Tropical	Yield potential	44	24	20
Nigeria/IITA	Drought and striga	5	3	2
Zimbabwe	Drought and low nitrogen	40	40	0

Table 2. Summary of trials conducted in Mexico, Kenya, Thailand and Zimbabwe under anthesis-stage drought stress, combined drought and heat stress, heat stress alone, and well-watered conditions. Mean and range for grain yield using best linear unbiased predictions (BLUPs), anthesis date, anthesis-silking interval (ASI) and plant height.

Location	Sowing date	Plot size (m <sup>2</sup> )	GY			Anthesis			ASI			PH		
			Mean	Range	H	Mean	Range	H	Mean	Range	H	Mean	Range	H
Drought														
Mexico	Nov 08	3.75	1.70	1.14-2.55	0.57	87.3	81.2-94.5	0.83	2.90	-0.33-11.7	0.49	173.4	144.5-195.8	0.51
Kenya	June 10	2.85	3.12	1.86-4.23	0.65	69.6	61.1-75.6	0.89	1.79	-2.53-12.07	0.49			
Mexico	Nov 09	7.50	2.67	0.89-5.52	0.80	87.4	77.3-94.9	0.95	2.87	-1.48-10.9	0.63	203.7	172.5-235.6	0.72
Thailand	Dec 09	7.50	2.35	1.30-4.09	0.58	57.1	52.3-61.7	0.92	5.11	-0.11-19.9	0.52	153.3	113.8-185.2	0.73
Mexico	Dec 10	7.50	3.30	1.94-4.55	0.69	82.2	73.1-90.0	0.94	2.09	-1.71-6.55	0.73	203.4	154.7-236.6	0.79
Kenya	Jun 11	2.85	2.22	0.85-3.36	0.47	66.1	55.0-74.0	0.92	3.25	-0.52-24.0	0.76	191.8	144.9-228.2	0.77
Thailand	Dec 10	7.50	2.80	1.30-4.14	0.69	57.9	53.5-62.9	0.92	2.81	-0.44-12.9	0.76	159.9	130.2-186.5	0.77
Combined drought and heat stress														
Mexico	Feb 10	7.50	0.24	0.14-0.64	0.32	71.9	67.1-91.9	0.81	12.01	-3.54-24.2	0.12	165.5	95.1-192.3	0.53
Zimbabwe			0.86	0.59-1.34	0.36	85.8	77.5-93.5	0.55	6.89	-2.69-25.4	0.53	142.9	86.1-171.3	0.26
Mexico	Feb 11	7.50	2.25	0.52-4.00	0.72	68.8	60.3-76.3	0.89	2.64	-1.06-6.65	0.66	184.2	94.0-211.7	0.72
Well-watered														

Mexico	Nov 08	3.75	7.30	5.26- 9.30	0.80	87.3	79.6- 93.9	0.83	1.44	-1.67- 8.67	0.40	189.2	139.3- 223.8	0.75
Mexico	Nov 09	7.50	7.39	5.24- 9.85	0.76	79.9	70.9- 87.5	0.85	2.49	-2.50- 5.50	0.53	210.6	157.4- 280.8	0.90
Thailand	Dec 09	7.50	6.50	4.40- 8.19	0.76	59.4	53.5- 64.0	0.63	0.58	-2.00- 3.00	0.43	170.8	133.5- 213.7	0.86
Mexico	Nov 10	7.50	7.38	5.87- 8.62	0.54	87.1	76.8- 94.7	0.93	2.03	-2.30- 7.51	0.63	219.5	175.2- 262.6	0.80
Thailand	Dec 10	7.50	6.50	4.40- 8.19	0.76	59.4	53.5- 64.0	0.86	0.58	-2.00- 3.00	0.73	170.8	133.5- 213.7	0.82
Heat														
India	March 11		1.50	0.55- 2.98	0.64	58.6	51.0- 65.9	0.73	6.19	-2.02- 18.2	0.18	129.7	94.4- 162.2	0.45

Table 3. Estimated variance components ( $\sigma^2$ ) for grain yield from the combined ANOVA across locations for both drought stress and well-watered trials. All estimates are calculated from standardized data.

	Well-watered	Drought stress	Combined drought and heat stress
$\sigma_g^2$	0.35	0.12	0.07
$\sigma_{g \times e}^2$	0.24	0.36	0.12
$\sigma_e^2$	0.48	0.39	0.18
No of locations	7	7	3
H	0.84	0.64	0.50

Table 4. Genetic correlations for grain yield between combined means for drought, combined drought and heat, heat and well-watered conditions.

	Drought	Combined drought and heat stress	Heat	Well-watered
Drought	-			
Combined drought and heat stress	0.08	-		
Heat	0.49	-0.07	-	
Well-watered	0.63	0.24	0.27	-

Table 5. Genetic correlations for gain yields between individual experiments in Kenya, Mexico, Thailand and Zimbabwe under drought, combined drought and heat, well-watered and heat stress.

			Drought					Combined drought and heat			Well-watered					
			2009 Mexico	2010 Kenya	2010 Mexico	2010 Thai- land	2011 Kenya	2011 Mexico	2011 Thai- land	2010 Mexico	2010 Zim- babwe	2011 Mexico	2009 Mexico	2010 Mexico	2010 Thai- land	2011 Mexico
Drought	2009	Mexico	1													
	2010	Kenya	<b>0.36**</b>	1												
	2010	Mexico	<b>0.14*</b>	<b>0.59**</b>	1											
	2010	Thai- land	<b>0.27**</b>	<b>0.51**</b>	<b>0.21**</b>	1										
	2011	Kenya	<b>-0.30**</b>	-0.01	<b>0.48**</b>	0.03	1									
	2011	Mexico	0.02	<b>0.22**</b>	<b>0.57**</b>	<b>0.25**</b>	<b>0.95**</b>	1								
Drought and heat	2011	Thai- land	<b>0.30**</b>	<b>0.38**</b>	<b>0.24**</b>	<b>0.72**</b>	0.01	<b>0.39**</b>	1							
	2010	Mexico	<b>0.58**</b>	<b>0.42**</b>	<b>0.12*</b>	<b>0.11*</b>	<b>0.35**</b>	-0.05	<b>0.28**</b>	1						
	2010	Zim- babwe	<b>0.40**</b>	<b>0.21**</b>	<b>-0.25**</b>	<b>0.16**</b>	<b>0.48**</b>	<b>-0.36**</b>	<b>0.33**</b>	<b>0.43**</b>	1					
	2011	Mexico	<b>0.58**</b>	<b>0.34**</b>	<b>0.14*</b>	<b>0.21**</b>	<b>0.58**</b>	<b>-0.20**</b>	<b>0.0**</b>	<b>0.73**</b>	<b>0.52**</b>	1				
Well-watered	2009	Mexico	<b>0.53**</b>	<b>0.29**</b>	<b>0.62**</b>	<b>0.09**</b>	0.01	<b>-0.18**</b>	<b>0.11*</b>	<b>0.3**</b>	<b>-0.13*</b>	<b>0.3**</b>	1			
	2010	Mexico	0.09	<b>0.41**</b>	<b>0.79**</b>	<b>0.18**</b>	0.01	0.01	<b>0.22**</b>	<b>0.22**</b>	<b>-0.13*</b>	<b>0.3**</b>	<b>0.6**</b>	1		
	2010	Thai- land	<b>0.24**</b>	<b>0.34**</b>	<b>0.35**</b>	<b>0.58**</b>	-0.05	-0.06	<b>0.49**</b>	<b>0.12*</b>	<b>0.22**</b>	<b>0.25**</b>	<b>0.68**</b>	<b>0.65**</b>	1	
	2011	Mexico	0.09	<b>0.41**</b>	<b>0.79**</b>	<b>0.19**</b>	<b>0.19**</b>	<b>0.24**</b>	<b>0.21**</b>	0.02	<b>-0.17**</b>	<b>0.19**</b>	<b>0.72**</b>	<b>0.66**</b>	<b>0.46**</b>	1
	2011	Thai- land	-0.06	<b>0.26**</b>	<b>0.45**</b>	<b>0.34**</b>	<b>0.26**</b>	<b>0.47**</b>	<b>0.52**</b>	-0.05	0.06	0.03	<b>0.76**</b>	<b>0.49**</b>	<b>0.9**</b>	<b>0.57**</b>

at	2011	India	<b>0.25**</b>	<b>0.22**</b>	<b>0.48**</b>	<b>0.05**</b>	<b>0.36**</b>	<b>0.28**</b>	<b>0.19**</b>	0.01	<b>-0.24**</b>	0.03	<b>0.34**</b>	<b>0.35**</b>	<b>0.29**</b>	<b>0.30**</b>	<b>0.34**</b>
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Table 6. Top 10 lines in combination with CML539 under drought and heat stress. Best linear unbiased predictions for grain yield under drought (DS), combined drought and heat stress (DS + H) and well-watered (WW) treatments, and best linear unbiased estimates for anthesis–silking interval (ASI) and heat stress (H) are presented.

Entry	Pedigree	Grain yield (t ha <sup>-1</sup> )						ASI			
		DS	DS + H	Rank	H	Rank	WW	DS	DS + H	H	WW
2	[SYN-USAB2/SYN-ELIB2]-12-1-1-2	3.22	0.81	296	2.08	49	6.98	2.1	5.8	4.4	1.3
251	La Posta Seq C7-F18-3-2-1-1	3.16	1.13	151	2.40	26	7.04	0.8	7.3	4.2	0.5
217	DTPWC9-F24-4-3-1	3.10	1.43	16	1.47	134	6.97	0.5	3.3	6.0	1.0
269	DTPWC9-F2-3-2-1	3.10	0.95	256	2.15	41	6.67	2.1	8.8	4.9	1.0
190	CLQ-RCYQ40 = (CML165 x CLQ-6203)-B-9-1-1	3.10	0.98	236	2.81	12	6.72	2.6	10.4	3.6	2.1
237	DTPYC9-F46-1-2-1-1	3.07	1.23	86	0.37	263	6.73	0.8	4.4	5.3	0.8
238	DTPYC9-F46-1-2-1-2	3.07	1.58	4	1.52	126	7.12	0.4	1.6	5.0	1.0
103	POB.502 c3 F2 10-3-2-1	3.07	1.17	125	2.99	9	7.54	2.1	3.9	2.1	1.5
	La Posta Seq C7-F64-2-6-2-2	3.06	1.48	7	-	-	7.65	-0.9	6.9	-	0.9
261	La Posta Seq C7-F180-3-1-1-1	3.03	1.13	149	0.77	233	7.94	1.0	10.6	8.5	0.3
Check	CML444	2.41	-	-	-	-	7.15	11.7	-	-	3.1
Check	CML444/CML442	2.36	0.93	169	1.35	155	7.39	2.8	5.8	4.1	0.5
	Trial mean	2.58	1.13	-	2.58	-	6.88	3.0	7.2	6.2	2.0
	LSD <sub>0.05</sub>	0.94	0.78	-	0.94	-	1.16	2.3	7.9	9.2	1.7

Table 7. Top 10 lines in combination with CML539 under combined drought and heat stress. Best linear unbiased predictions for grain yield under drought (DS), combined drought and heat stress (DS + H) and well-watered (WW) treatments, and best linear unbiased estimates for anthesis–silking interval (ASI) and heat stress (H) are presented.

Rank	No	Pedigree	Grain yield (t ha <sup>-1</sup> )					ASI	
			DS+H	DS	Rank	WW	DS + H	DS	WW
1	257	La Posta Seq C7-F86-3-1-1-1	1.60	2.81	60	7.83	4.2	2.3	1.3
2	207	CL-G1628=G16BNSeqCOF118-1-1-4-2	1.60	2.81	61	6.77	3.4	2.5	3.6
3	258	La Posta Seq C7-F96-1-6-2-1	1.58	2.35	265	7.59	2.9	1.2	1.3
4	238	DTPYC9-F46-1-2-1-2	1.58	3.07	7	7.12	1.6	0.4	1.0
5	52	CLA135	1.50	2.56	173	6.75	2.5	2.1	1.5
6	191	CLQ-RCYQ28=(CLQ6502*CLQ6601)-B-34-2-2	1.49	2.45	223	7.26	6.8	4.1	3.0
7		La Posta Seq C7-F64-2-6-2-2	1.48	2.90	9	7.65	1.6	0.8	0.9
8	248	La Posta Seq C7-F123-3-1-1-2	1.48	2.54	185	7.17	5.0	2.3	1.5
9	143	[CML-384 X CML-176](F3)100-2-7	1.48	2.71	97	7.44	6.5	3.4	2.9
10	125	[M37W/ZM607#bF37sr-2-3sr-6-2-X]-8-2-X-1-BB-B-xP84c1 F27-4-3-3-B-1-B] F29-1-2-1-4 x (87036/87923)-X-800-3-1-X-1-B-B-1-1-1-B-B-xP84c1 F26-2-2-4-B-2-B]-1-1-B x CML486]-1-1	1.47	2.58	161	6.93	2.6	2.4	2.7
	Check	CML444	-	2.41	171	7.15	11.7	-	3.1
	Check	CML444/CML442	0.93	2.36	261	7.39	2.8	5.8	0.5
	Trial mean		1.13	2.58		6.88	3.0	7.2	2.0
	LSD <sub>0.05</sub>		0.78	0.94		1.16	2.3	7.9	1.7



