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Identification and Performance of Early Maturing Pro-vitamin A (PVA) Maize under Combined Drought and Heat Stress

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Abstract: *The impact of climate change on maize production and low maize yields highlights the crucial need to improve yield in drought prone zones in West and Central Africa (WCA). Climate change projections suggest higher temperatures within the areas. Therefore, there is need to identify and develop maize cultivars tolerant to combined drought and heat stress (CDHS). An increase in temperature above 30^{o}C reduces yield by 1% under optimal (rain-fed) condition, 1.7% under drought stress (DS) and up to 40 % under CDHS. The objective of this study is to identify traits that contributed to better performance of maize yield under CDHS. Two hundred and six early maturing PVA hybrids and four commercial checks were used for evaluation inKadawa Nigeria for two years.Highly significant (p < 0.001) differences were found among hybrids for grain yield and other measured agronomic traits. Moderately high to low heritability values were detected for grain yield under CHDS (0.46) and under optimal condition (0.85). Hybrids TZEIOR 172 x TZEIOR 108 and TZEIOR 202 x TZEI 25 were tolerant to CDHS and were high-yielding and stable across environments having the potential for improving nutrition and maize yields in SSA. These hybrids could be further tested for possible release.*

Keywords: *combined drought and heat stress; PVA; grain yield; maize; climate change;hybrid*

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I. Introduction

Deficiency in Vitamin A poses health risks to children, pregnant women, and nursing mothers in West and Central Africa (WCA). Maize cultivars biofortified with Provitamin A can contribute to minimizing the adverse effects of vitamin A deficiency (VAD) in areas where maize is a staple food crop. It has been estimated that 50 % of the total population of WCA depends on maize as a staple food, raw materials for various alcoholic beverages, poultry and the livestock industries (Badu-Apraku*et al.,* 2017). As a highly consumed cereal of the sub-region, maize plays an important role in combating malnutrition. The vulnerability of maize is high under drought and heat stress during reproductive stages, drought stress at flowering and grain-filling stages of maize causes delayed silking, increased anthesis-silking interval, and reduced kernel set resulting in grain yield (GY) losses above 50% (Cairns *et al*., 2013; Badu-Apraku*et al*., 2017). Currently, due climate change effects farmers in drought prone agro-ecological zones of SSA are not willing to accept maize hybrids that are without combined tolerance/resistance to multiple stresses (Benjamin *et al*., 2019). The development and commercialization of PVA maize hybrids with early maturity (90–95 days) is therefore very important for sustainable maize production in WCA and helps in combating malnutrition. According to Olatunde*et al*.,2021, 40 % of the maize production areas in SSA will be unsuitable for cultivation of the available maize germplasm due to the threat posed by combined drought and heat stress(CDHS) by 2030. A major challenge of maize breeders of the present generation is to develop maize cultivars with CDHS tolerance for the agroecological zones of sub-Sahara Africa (SSA).

II. Material and Methods

All genetic materials were sourced from the International Institute of Tropical Agriculture (IITA)-Maize Improved Program (MIP) unit. The development of the PVA-QPM inbred lines was initiated in 2007 by crossing a drought and *Striga* resistant early QPM variety, TZE-Y-Pop-DT-STR-QPM with an intermediate maturing (105-110 days to maturity) high PVA maize [Syn-KU1409/DES/1409-(OR2)] from the IITA-MIP to introgress genes for high β-carotene into the QPM variety. This was followed by a cycle of backcrossing to the recurrent parent to recover earliness. In 2008, the BC_1F_2 lines with deep orange colour (for PVA) and/or appropriate endosperm modifications were selected and advanced to the F_2 and the F_3 generations. In 2009, the F_3 lines were selected based on their reactions to *Striga* and drought, then recombined to reconstitute the early PVA-QPM variety, 2009 TZE-OR2-DT-STR-QPM. Subsequent evaluations of this variety for drought tolerance in 2010 showed superior performance. As part of another programme initiated in 2011 to extract the first generation of early maturing inbred lines from different high PVA sources, S_1 lines from the PVA-QPM variety 2009-TZE -OR2 DT-STR QPM were advanced through inbreeding to the S_6 generation from 2011 to 2014. During the inbreeding programme, the inbred lines were screened at the S_2 to the S_5 generations to select kernels with deep orange colour for the PVA trait and the appropriate endosperms modifications ranging from 25 – 50 % opaqueness for the QPM trait. This study evaluated 250 PVA-QPM inbred lines andsix PVA-QPM inbred checks. In addition, fiftyselected inbred lines based on the physiological performancealongside four inbred testers (TZEIOR 108, TZEIOR 164, TZEI 25, and TZEI 129 which were intercrossed to obtain six single-cross hybrids)were added to four commercial hybrid checks to make a total of two hundred and ten single-cross hybrids for the genetic study using the line x tester mating design. The 210 hybrids were subsequently evaluated under combined drought and heat stress and optimal environments at Kadawaduring the 2019, 2020 and 2021wet growing seasons in Nigeria.

A 14 x 15 randomized incomplete block design with two replicates were used for all hybrid evaluations a 16 × 16 randomized incomplete block design with two replicates was used for all inbred line evaluations. Single-row plots, 3 m long, with a spacing of 0.75 m between two adjacent rows and 0.40 m between plants within rows were used for both the inbred line and hybrid evaluations. Three seeds were sown per planting hole and the emerged seedlings thinned to two per stand at 2 weeks after emergence, resulting in a final plant population density of about 66,667 plants ha^{-1}. Pre-emergence weeds were controlled by applying gramoxone and atrazine at the rates of 1.5 L gramoxone and 2.5 L atrazine in 200 L of water ha^{-1} under CDHS and optimal evaluations. In the first set of trial evaluation at Kadawa (11°45' N, 8°45' E, 468.5 m ASL, 884 mm rainfall) during 2020 and 2021 dry seasons in Nigeria, where drought stress at elevated temperature occur between February and June yearly. The soil type at Kadawa is characterized as Regosols, with mainly sandy to clay loam texture. The trials were irrigated twice weekly for the first 28 days after planting using a furrow irrigation system there after the plants rely on water stored in the soil. The trials were subjected to CDHS for three weeks during the month of April where day temperature ranges from 35°C to 40°C and night temperature varied from 22 °C to 28 °C.

Procedure methodology

Data was recorded under each trial for 50 % days to anthesis (DA), 50 % days to silking (DS), anthesis-silking interval (ASI) calculated as the difference between DA and DS, plant and ear heights (PLHT and EHT) were measured as the distance from the base of the plant to the height of the first tassel branch and the node bearing the upper ear, respectively, root and stalk lodging (RL and SL), RL (percentage of plants leaning more than 30◦ from the vertical), SL (percentage broken at or below the highest ear node) were recorded few days to harvest, At 70 DAP, plant and ear aspects (PASP and EASP) were scored, PASP was scored based on the general architectural appeal of plants in a plot (standability, vigour, plant and ear height, uniformity of plants, ear placement and size, as well as disease damage and lodging) using a scale of 1 to 9, where 1 = excellent overall phenotypic appeal and 9 = completely undesirable phenotypic appeal, EASP was recorded based on general appeal of the ears without the husks (ear size and number; uniformity of size, colour and texture; extent of grain filling, insect and disease damage) using a scale of 1 to 9, where 1 = excellent (clean, uniform, large, and fully filled ears with no disease/insect damage) and 9 = only one or no ears. Similarly, husk cover (HC) was rated on a scale of 1 to 5, where 1 = husks tightly arranged and extended beyond the ear tip and 5 = exposed ears. At harvest, number of ears per plant (EPP) was obtained by dividing the total number of ears per plot by the total number of plants harvested. Additional data recorded under CDHS were stay-green characteristics scored at 70 DAP on a scale of 1 to 9 where, 1= all leaves are green and 9 = all leaves are dead, tassel blast and leaf firing were recorded at flowering stage on a scale of 1 to 9, where 1 = all plants had normal pollen production and 9 = all plants had white, dried tassels without pollen production. Grain yield (kg ha^{-1}) for optimal was estimated from field weight of ears per plot, assuming a shelling percentage of 80, adjusted to moisture content of 15 %. Moisture content at harvest was recorded for representative shelled kernels per plot in all experiments using a moisture meter.

Statistical Analysis

Data on grain yield and other agronomic traits were subjected to combined ANOVA for each environment using PROC GLM in Statistical Analysis System (SAS) version 9.3 (SAS Inc. 2011) to obtain mean squares for each trait. Analysis of variance were performed on the adjusted means of the individual traits under combined drought and heat stress and optimal growing conditions and thereafter combined across environments. Subsequently, combined ANOVA was performed across the seven test environments. In the ANOVA for each and across environments, the environments, replications within environments, and incomplete blocks within replications × environment interaction were considered as random factors whereas the entries (inbreds) were regarded as a fixed factor. The statistical model corresponding to the experimental layout was:

$$y_{klmi}= \mu_i+ E_{ki}+ R(E)_{kli}+G_{mi}+GE_{kmi}+\epsilon_{klmi}$$

where y_{klmi} is the observed measurement of trait $i$with mean effect μ_i. E_{ki} is the effect of environment k on trait i, $R(E)_{kli}$ is the effect of replication l within environment k on trait i, G_{mi} is the effect of genotype m on trait i, GE_{kmi} is the effect of the interaction between genotypes m and environment k on trait i, and ϵ_{klmi} is the experimental error effect associated with genotype m and replication l within environment k on trait i. The entry means were adjusted for block effects, according to the lattice design (Cochran and Cox, 1960) and means were separated using the standard error (S.E).

Inter-Trait Relationships under CDHS and Optimal growing Environments

The step-wise multiple regression and sequential path diagrams were employed to determine the causal relationships between the measured traits under CDHS and optimal growing environments using the procedure described by Olatunde*et al.,* 2021. The step-wise multiple regression analysis was done using the Statistical Package for the Social Sciences, SPSS v. 17.0 to determine the first, second,and thirdorder predictor traits on the basis of their contributions to grain yield variation. The secondary traits were regressed on grain yield to identify first order traits that contributed significantly to grain yield at $P \leq 0.05$. The remaining secondary traits were regressed on the first order traits to identify those with significant contributions to grain yield and they were categorized as second order traits. The procedure was repeated in order to categorize the remaining traits into subsequent orders. The standardized b values generated by the step-wise regression analysis were the path coefficients (Mohammadi*et al*., 2003). The significance of a path coefficient was determined in the stepwise regression analysis using the t-test with a probability level of 5% and only traits with a significant path coefficients were retained. In addition, spearman correlation analysis implemented in SAS v.9.4 was done to determine the relationships among traits within the same order.

III. Results

Analysis of Variance of Measured Agronomic Traits

Under the CDHS environments, analysis of variance (ANOVA) indicated significant ($P < 0.05$) variations among Environment(E), Genotype (G),genotype by environment interaction(GEI) and mean square for grain yield and all measured traits except GEI for ear aspect (EASP), ears per plant (EPP), ear height (EHT) and stay green characteristics (STGR). Broad sense heritability (H^2) estimates ranged from 28 % for leaf firing to 65 % for stay green characteristics. Grain yield recorded H^2 estimate of 58 %. Overall, low to high H^2 estimates were observed for the agronomic measured traits under CDHS.

Across the optimal environments, significant ($P < 0.05$) differences were observed among Environment (E), Genotype (G), and genotype by environment interaction (GEI), mean squares for all measured traits. Similarly, significant differences were observed for all measured traits under G except for EPP and plant height (PLHT). Estimates of broad sense heritability varied from 65 % for 50 % days to anthesis (DA) to 76 % for PLHT. Grain yield recorded H^2 estimate of 88 %. High to very high H^2 estimates were observed for the measured traits, with no exception.

Table 1: Mean squares from analysis of variance for grain yield and other agronomic traits of 210 early maturing pro-vitamin A maize hybrids and four checks evaluated under combined drought and heat stress at Kadawa (2020 and 2021) in Nigeria

SOURCE	DF	GY (t ha^{-1})	DA	DS	ASI	EASP	HUSK	EPP	PLHT (cm)	EHT (cm)	STGR	TB	LF
ENV	1	8410866**	39.87**	259.63**	108.58**	998.88**	641.38**	9.85**	20041.20**	8924.58**	2.63	8.4**	0.80
Entry	209	7580051**	7.97**	9.35**	0.91	2.46**	0.90	0.039	477.33**	223.99**	1.23**	0.43	0.64**
Rep(ENV)	2	1460304**	0.51	0.95	0.12	31.93**	39.44**	0.03	376.53	544.95**	66.51**	0.15	10.10**
BLK(ENV*Rep)	56	1766226	10.47**	12.15**	0.95	4.90**	2.55**	0.05**	705.91**	220.75**	2.89**	0.62**	0.53
Entry*ENV	209	858395	9.86**	12.30**	1.12**	2.09	0.91**	0.03	211.88	101.43**	1.01	0.46**	0.35**
Error	362	1882354	2.61	3.30	0.80	1.77	0.73	0.03	157.92	82.87	0.87	0.38	0.40
Heritability	-	**58**	**51**	**55**	**60**	**61**	**50**	**42**	**60**	**52**	**65**	**62**	**28**

*, ** = Significant at 0.05 and 0.001 probability levels, respectively; Env = environment; Rep = replication; GY = Grain yield; DA = days to 50 % anthesis; DS = days to 50 % silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; PASP = plant aspect; EASP = ear plant aspect; EPP = ears per plant; STGR = stay green characteristic; TB = tassel blast; LF = leaf firing.

Table 2: Mean squares from analysis of variance for grain yield and other agronomic traits of 210 early maturing pro-vitamin A maize hybrids and four checks evaluated under optimal conditions at Mokwa (2019 and 2020) and Kadawa (2020) in Nigeria

SOURCE	DF	GY (t ha^{-1})	DA	DS	ASI	PASP	EASP	EPP	PLHT(cm)	EHT(cm)
ENV	2	609772240.00**	3257.01**	5047.49**	421.67**	284.89**	590.21**	2.29**	478162.79**	164364.12**
Entry	209	4121951.00**	8.20**	8.45**	0.94**	1.64**	4.66**	0.06	448.74	276.68**
Rep(ENV)	3	114006685.00*	952.83**	1206.87**	8.69*	23.97**	77.03**	0.24**	47981.23**	17055.44**
BLK(ENV*Rep)	84	5558521.00**	22.14**	27.44**	0.86**	1.69**	4.43**	0.08**	1439.51*	626.89**
Entry*ENV	414	2142755.00**	4.02**	4.83**	0.83*	0.88*	2.92*	0.06**	259.78**	157.50**
Error	539	1917068.00	3.28	4.33**	0.82	0.73	2.76	0.04	160.77	123.06
Heritability	-	**88**	**65**	**70**	**65**	**70**	**60**	**75**	**76**	**50**

*, ** = Significant at 0.05 and 0.001 probability levels, respectively; Env = environment; Rep = replication; GY = Grain yield; DA = days to 50 % anthesis; DS = days to 50 % silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; PASP = plant aspect; EASP = ear plant aspect; EPP = ears per plant.

Across environments (table 3), significant ($P < 0.01$ or $P < 0.05$) differences were observed among Environment (E), Genotype (G), and genotype by environment interaction (GEI) mean squares for all measured traits except ears per plant (EPP) and husk cover (HUSK) for GEI. The mean squares under research conditions were significant ($P < 0.01$) for measured traits except for plant height (PLHT). Moderate to high broad sense heritability estimates were recorded for the measured traits across environments, varying from 34 % for anthesis to silking interval (ASI) to 91 % for ear aspect (EASP). Grain yield had a moderately high H^2 estimate of 68 %.

Table 3: Mean squares from analysis of variance for grain yield and other agronomic traits of 210 early maturing pro-vitamin A maize hybrids and four checks evaluated under optimal condition and combined drought and heat stress in Kadawa between 2019 and 2020 growing seasons

SOURCE	DF	GY	DA	DS	ASI	EASP	EPP	PLHT	EHT	HUSK
ENV	2	769712222.00**	23118.41**	27592.27*	207.34**	94.23**	0.71**	224900.62**	28171.34**	263.30**
Entry	209	3747714.00**	5.98**	7.22**	0.96**	1.77**	0.04*	453.70	225.72**	0.31**
Rep(ENV)	3	15878985.00**	25.16**	30.36**	0.71**	7.90**	0.51**	2746.26**	164.25**	1.15**
BLK(ENV*Rep)	84	4823388.00**	6.63*	8.28**	1.26**	2.64**	0.07**	877.30*	291.48**	0.68**
Entry*ENV	418	3057433.00**	6.71**	8.25**	0.94**	1.10*	0.05	334.38*	165.36**	0.28
Error	542	1812511.00	2.38	3.12	0.82	0.90	0.04	254.99	131.05	0.20
Heritability	**-**	**68**	**54**	**62**	**34**	**91**	**72**	**67**	**73**	**74**

*, ** = Significant at 0.05 and 0.001 probability levels, respectively; Env = environment; Rep = replication; GY = Grain yield; DA = days to 50 % anthesis; DS = days to 50 % silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; EASP = ear plant aspect; EPP = ears per plant.

Step-Wise Multiple Regression and Sequential Path Analyses

Under combined drought and heat stress, ear aspect, ears per plant, stalk lodging and stay green characteristicswere identified by the stepwise multiple regression analysis as first order traits, withsignificant contributions to grain yield, explaining 90 % of the total variation in grain yieldoftheearlymaturingPVAhybrids (Figure 1). Ofthefourfirstordertraits,earaspect hadthehighestnegativedirecteffect(-0.597)ongrainyield,whilealowerdirectcontributionof0.036wasmadebystalklodging.Positivedirectcontributionswe remadebyears per plant (0.413) and stay green characteristics (0.036). Five traits recorded indirect contributionstograinyieldthroughoneortwoofthefirstordertraitsandwerecategorizedastraitsin the second order. Among the five traits, days to 50 % days to anthesis and plant aspect indirectly contributed to grainyieldthroughtwoofthefirstordertraits,likewise days to 50 % silking.Eachoftheremainingsecondorder traits, days to 50 % silking and plant height made theircontributionstograinyieldthroughafirstordertrait. Days to 50 % anthesismadethehighestnegative(-0.416)indirect contribution to grain yield through ears per plant, while plant aspect made thehighest positive indirectcontribution to grainyield through ear aspectunder drought.Furthermore, each of the three third order traits (ear height, ear rot and root lodging)madeindirectcontributionstograinyieldthroughallthefivesecondordertraitsexceptrootlodging which contributed to grain yield through three of the first order traits.

Under optimal conditions (figure 2), plant aspect, ear aspects, ears per plant and ear rot had directcontributions to grain yield with 93 % of the variation in grain yield of the early maturingPVA-QPMhybridsattributedtothesetraits.Plantandearaspectsandearrotmadesignificant negative and direct contributions, while ears per plant made a positive directcontribution to grain yield. Ear rot as a first order trait had a negative contribution to grainyield. Husk cover indirectly contributed to grain yield through ear rot.

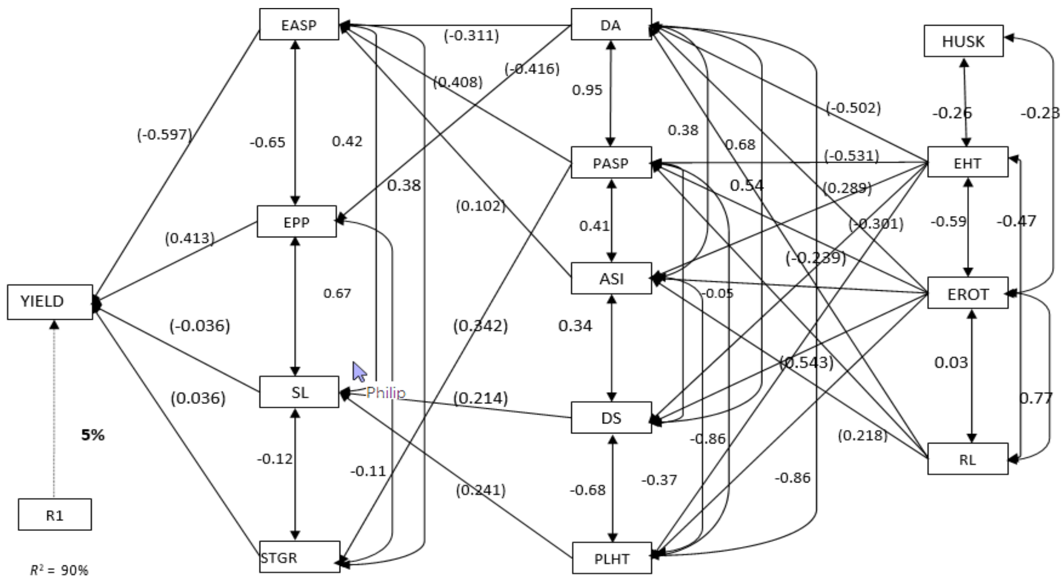

Figure 1: Path analysis model diagram showing causal relationships of measured traits of early maturing provitamin A maize hybrids evaluated under combined drought and heat stress during the 2020 and 2021 dry seasons at Kawada in Nigeria. Bold valueis the residual effect; values in parenthesis are direct path coefficients while other values are correlation coefficients. R^2= co-efficient of determination; R1= residual effects; YIELD= grain yield;EASP=earaspect;EPP=earsperplant;DA=daysto50%anthesis;SL=stalklodging;ASI=anthesis–silkinginterval;PASP=plantaspect;DS=daysto50%silking;STGR=staygreencharacteristics;PLHT=plant height; EHT=ear height; HC=huskcover; RL=rootlodging; EROT=ear rot

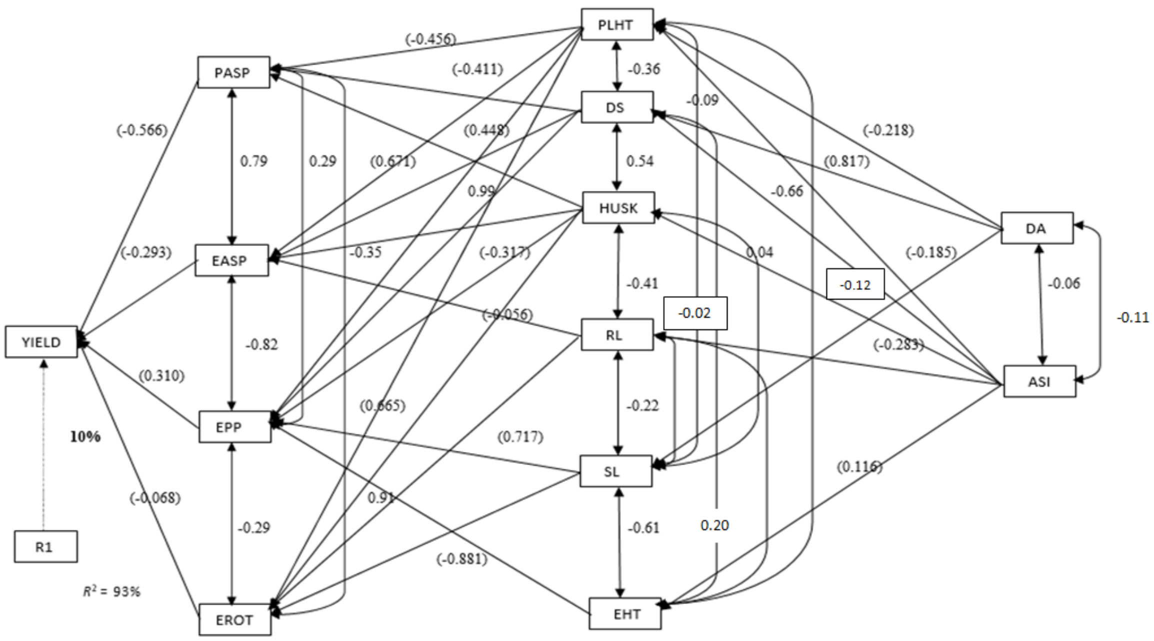

Figure 2: Path analysis model diagram showing causal relationships of measured traits of early maturing provitamin A maize hybrids evaluated under optimal environments in the 2019, 2020 and 2021 growing seasons at Mokwa in Nigeria. Bold value is the residual effect; values in parenthesis are direct path coefficients while other values are correlation coefficients. R^2= co-efficient of determination; R1= residual effects; YIELD= grain yield; PASP= plant aspect; EASP= ear aspect; EPP= ears per plant; EROT= ear rot; PLHT= plant height; DS= days to 50 % silking; HUSK= husk cover; EHT= ear height; SL= stalk lodging; DA= days to 50% anthesis; ASI= anthesis–silking interval; RL= root lodging.

IV. Discussion

The significant differences observed among measured traits under combined drought and heat stress and optimal environments indicated that the germplasms evaluated in this study are genetically diverse whichshould allow good progress from selection under the contrasting environments. The significant environmental variation for all traits under combined drought and heat stress and optimal environments indicated that each environment was unique and highly variable emphasizing the need for testing in more environments over years.This present study has shown large genetic variation in grain yield under combined drought and heat stress in maize germplasm on the field. Research is on-going as regards the extensive breeding effort that targets specifically combined drought and heat stress in tropical and sub-tropical regions. Several potential donors with tolerance to combined drought and heat stress were identified in this study, however further trials are needed to confirm these results over several years.

Combined drought and heat stress is likely to become an increasing constraint to maize production in SSA, particularly in the drought-prone lowlands of Africa (Cairns *et al*. 2012). When considering the importance of identifying maize genotypes with high levels of tolerance to combined drought and heat stress for climate change adaptation, promising drought tolerant hybrids were screened under combined drought and heat stress (CDHS). The significant differences found among the hybrids for grainyield and several other measured agronomic traits recorded under CDHS, indicated the presenceof genotypic difference among promising hybrids. The significant interactions of hybrids withenvironments for grain yield and other measured agronomic traits suggest the influence of CDHS onthe expression of these traits. The greatest influence of CDHS occurred in mid-April ofeach year, during the three weeks around flowering and grain-filling stages, these two stages are themost critical period determining yield potential in maize production.

The extent of yield loss is dependent on severity of drought and heat stress, field environmentand the maize genotypes under study. In this study, combined drought and heat stress reduced grain yield by 74 %, agreeing with previous findings that demonstrated moreyield loss from combined effects of drought and heat stress than drought stress alone (Lobell *et al.*, 2011).Combined drought and heat stress adversely affected grain yield and days to flowering of mosthybrids more than drought, possibly due to its adverse effects on pollen production, or ovule fertility, leading to premature embryo abortion and reduced grain weight (Meseka*et al.*, 2018).Some combined drought and heat stress (CDHS)hybrids that produced high grain yields under CDHS were found in the present study, implying that in a short term, breeders can commercialize new maize germplasm that are tolerant to CDHS quickly after further testing has been done as well as for their use as donors of CDHS. The observed significant correlations between the same traits measured under CDHS as well as optimum conditions suggest the presence of common genetic factors controlling expression of these traits under the two growing conditions.

V. Conclusion

The savanna agro-ecologies of WCA have the highest potential for maize grain yield production and productivity due to low night temperatures, low incidence of pests and diseases and high solar radiation, all of which favour maize production and productivity. However, declining soil fertility, heat stress, drought stress and recently combined drought and heat stress are the major limiting factors to maize production and productivity in the savannas of the subregion. These stresses, when present together, drastically reduce maize production. Genetic enhancement of maize under drought stress and/or heat stress could improve grain yield of farmers.The significant environmental and genotypic variations observed among the inbred lines across combined drought and heat stress environments for grain yield and the other measured agronomic traits indicated that the environments were unique and significant progress could be made in selecting lines tolerant to combined drought and heat stress. In terms of nutritive value, PVA maize can provide more than 15 $\mu g\ g^{-1}$ dry weight of PVA to combat vitamin A deficiency (VAD) and its health related problems.

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