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Journal of
**Plant Breeding
and Crop Science**

June 2018
ISSN 2006-9758
DOI: 10.5897/JPBCS
www.academicjournals.org



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Full Length Research Paper

Inheritance and combining ability study on drought tolerance and grain yield among early maturing inbred lines of maize (*Zea mays* L.)

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Received 1 November, 2017; Accepted 8 January, 2018

Utilization of stress tolerant maize is key to sustainable production and food security. Limited knowledge on genetics of drought tolerance hampers development of drought tolerant varieties. The aim of this study was to study inheritance and combining ability of drought tolerance and grain yield among early inbred lines of maize. Five inbred lines were mated in full diallel in 2015 major season. The resultant 20 hybrids, 5 parents and 2 checks were evaluated under drought stress and well watered conditions in a screen house. Data was collected on days to 50% tasseling (DT 50%), days to 50% silking (DS 50%), anthesis-silking interval (ASI), leaf rolling, leaf senescence, plant aspect (PASP), plant height (PHT), ear height (EHT), ears per plant (EPP), ear weight, hundred grain weight (100 GW) and grain yield per hectare (GY). Drought condition was induced 40 days after planting (DAP) but watering was continued once per week till maturity. Results from combined ANOVA showed there was high and significant ($p < 0.01$) level of genetic variability among parental lines and hybrids used in all the traits studied except DT 50%, PHT, EHT, EPP and 100 GW. Both additive and non-additive gene actions were important as well as GCA/SCA ratio variance. Lines TZEI-23 (215.22) and TZEI-25 (76.84) had the highest and highly significant ($p < 0.01$) positive GCA effects for GY under drought. Hybrids TZEI-25 x TZEI-13 showed the highest positive and highly significant ($p < 0.01$) SCA effects for GY (385.74) followed by its reciprocal TZEI-13 x TZEI-25 (311.49) under water stress. High broad sense heritability was observed for almost all the traits. High narrow sense heritability were observed DT 50% (0.69), DS 50% (0.80) and leaf senescence (0.61) under drought condition. Eighteen and nineteen hybrids had positive high parent heterosis (HPH) under water stress and well watered conditions, respectively for GY. The parents TZEI-23 and TZEI-25 were identified as the best general combiners respectively under drought and well watered conditions. The highest HPH were observed in many traits for TZEI-13 x TZEI-25 and TZEI-124 x TZEI-13. It is recommended these hybrids are further evaluated in different environments for release to farmers to increase yield.

Key words: Maize, inheritance, combining ability, drought, tolerance.

INTRODUCTION

Maize (*Zea mays* L.; $2n = 20$) belongs to the family Gramineae and tribe Maydeae or by others

Andropogoneae (Norman et al., 1995). It occupies the second position after wheat in terms of area of production

but represents the most important cereal crop in terms of quantity produced worldwide (FAOSTAT, 2017). In terms of consumption and cultivation, maize crop is very adaptable and versatile. In 2016, worldwide production of maize was around 1 billion ton, with America being the largest producer, which produces 51.6% equivalent to 547,416,865 tons and United States of America with 384,777,890 tons (FAOSTAT, 2017). Africa produces 6.7% and Nigeria is the largest African producer with 10,414,012 million tons (FAOSTAT, 2017). In 2016, Ghana produced 1,721,910 (FAOSTAT, 2017). According to Breisinger et al. (2008), maize is the most important cereal crop in Ghana in terms of consumption and production. In developed countries, maize crop is mainly used as an animal feed while it is largely used as a human consumption in developing countries (Badu-Apraku et al., 2012). In African, people consume maize as a starchy base in a wide variety of paste, beer grits and porridge. In sub-Saharan Africa, it is a staple food for around 50% of the total population (USAID, 2010). Maize crop is an important source of protein, minerals, vitamin B, iron, and carbohydrate (USAID, 2010).

As a result of continuous shortage and unpredictability of rains in the dried areas of the words, possibly due to the effect of changes in climatic (Sodangi et al., 2011), research attention is being directed toward producing maize hybrids that can withstand moisture stressed ecologies. Despite the level of adaptation that maize crop displays and its potential in savanna ecology, low yield are still obtained due to biotic and abiotic stresses. According to Kamara et al. (2004), drought and low soil fertility are among the most important stresses threatening food security, maize production and economic growth. In dry savanna zone of West Africa, drought effect on food supplies and maize crop production are most severe (Fajemisin et al., 1985), due to the unpredictable rainfall in the region in terms of establishment, quantity, and distribution (Izge and Dugie, 2011). In addition, recurrent drought is the single most important factor limiting maize production in West and Central Africa, with several billion U.S. dollars in production lost annually to this stress factor (Badu-Apraku et al., 2011). In Ghana, frequent drought stress is a major constraint in the largely rain-fed agricultural system that limits the production of maize (Mercer-Quashie et al., 1993; Ohemeng-Dapaah 1994; SARI, 1995; Obeng-Antwi et al., 1999). Drought stress has often been cited by farmers in Ghana as one of the major constraints to high maize productivity in the Coastal, Guinea and Soudan savanna zones in the major season and in the forest, coastal savanna and transition zones in the minor season.

Research attention should be directed toward maize hybrids production that can tolerate drought stressed ecologies. Predominant maize types cultivated by farmers in West Africa are open pollinated cultivars where hybrids accounted for just over 9%, OPVs for 41%, and local cultivars for 50% (Tsedeke et al., 2017). Therefore, the need for breeding maize crop tolerant to drought condition for high yield is important. International Institute for Tropical Agriculture (IITA) has developed a wide range of maize germplasm which are adapted to the climate conditions of sub-Saharan African countries (Laouali, 2014). A large number of their inbred lines were developed under stress conditions such as striga, drought and low nitrogen, and some of their early inbreds were used as source of germplasm in this study. However, information on combining abilities, heritability and heterosis of these inbreds is limited. Thus, information regarding combining abilities, heritability and heterosis is essential for selection of suitable parents for hybridization and identification of promising hybrids for the development of improved varieties for a diverse agro-ecology. Such information is useful in developing stable hybrid with high yield. Therefore, this study was conducted to estimate heritability and combining ability and determine performance of parents (inbred lines) and hybrids under water stress and well watered conditions among early inbred lines of maize.

MATERIALS AND METHODS

Experimental site and genetic materials

The experiment was conducted on the research field of the Department of Crop and Soil Sciences, KNUST, Kumasi, Ghana during the major season of 2015 (from April to July) to develop hybrids.

Five tropical inbreds maize, developed by IITA, which are adapted to the climate conditions of sub-Saharan African countries were obtained. These inbreds are TZEI-13, TZEI-17, TZEI-23, TZEI-25 and TZEI-124 and were used in full diallel 5 × 5 mating design. All possible crosses were done among five inbred lines.

The evaluation of the twenty diallel single crosses (Appendix 1) produce, the five inbreds parents and two checks (MAMABA and OMANKWA from Crop Research Institute, Kumasi Ghana) for drought tolerance was carried out in the screen house of the Department of horticulture, KNUST, Kumasi Ghana during the major season of 2016 (from March to June). The site is located geographically on latitude 06°40'N and 01°34'W of the Greenwich meridian. Sandy loam (sand 68.32%) is the physical property of the soil used in the screen house for the study. The pH of soil was 5.00 which indicates a very acidic soil condition.

Experimental design

A Completely Randomized Design (CRD) was used for the

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evaluation of the 27 genotypes under managed drought and well watered conditions. The treatments were replicated three times. Each plot contained three pots. Therefore a total of 486 potted plants were evaluated. The water stress and the well-watered experiments were planted in the same plant house in two adjacent blocks.

Planting, stress management and quantity of water applied

Planting was done on 13th March, 2016. Water was withdrawn at 40 day after planting (one week before tasseling) from the water stress regime but watering continue once in the week, while irrigation was kept normally till eleventh week after planting for the well watered condition which coincided with end of the grain filling.

Fertilizer application and pest and weed control

All management practices were the same in both water stress and well watered conditions. Hand weeding was done at tenth, twentieth and thirtieth DAP to control weeds. NPK (15:15:15) was applied at a rate of 5 g to each pot at twelfth DAP followed by urea, also the same rate (5 g) at 32nd DAP.

Data collection

Data were collected on both treatments. Days to 50% tasseling (DT 50%) and days to 50% silking (DS 50%) were determined as a number of days that 50% of plant showed tassels and 50% of plants extracted silks. Anthesis-silking interval (ASI) was calculated as the difference between days to 50% silking and days to 50% anthesis. Leaf rolling (Leaf R) was scored by using a scale from 1 to 5 (Bänziger et al., 2000), 1 = unrolled, turgid, 2 = leaf rim stars to roll, 3 = leaf has the shape of V, 4 = rolled leaf rim covers part of leaf blade, 5 = leaf is rolled like an onion. Plant aspect (PASP) was recorded on a scale of 1 to 5 based on overall plant type, where: 1 = excellent plant type (desirable plant and ear characteristics), 2 = very good plant type, 3 = good plant type, 4 = tolerable plant type and 5 = poor plant type (undesirable plant and ear characteristics). The score of leaf senescence (Leaf S) was recorded on the scale from 1 to 10 by dividing the percentage of estimated total dead leaf area by 10 (Bänziger et al., 2000) as 1 = 0-10% dead leaf, 2 = 10-20% dead leaf, 3 = 20-30% dead leaf, 4 = 30-40% dead leaf, 5 = 40-50% dead leaf, 6 = 50-60% dead leaf, 7 = 60-70% dead leaf, 8 = 70-80% dead leaf, 9 = 80-90% dead leaf and 10 = 90-100% dead leaf. Plant height (PHT) and ear height (EHT) were measured from the base of the maize plant to the top of the largest leaf and ear leaf, respectively using measuring tape (cm) (Badu-Apraku et al., 2012). Ears per plant (EPP) was recorded as the total number of ears which developed at least one full grain and divided by the total number of all the plants harvested in the plot (Bänziger et al., 2000). Ear weight was recorded by dividing the weight of total ears in the plot that at least exists one fully grain by number of harvested ears. After bulking the grain results of all plants within the plot, hundred grains were counted and weighted. The results were given in gram (g) by using SARTORIUS scale. Grain yield per hectare (GY) was calculated by adjusting the actual moisture level of grain. The conversion of grain yield to grain moisture-standardized yield was calculated as follows (Badu-Apraku et al., 2012):

$$\text{Yield (at 15\% grain moisture)} = \frac{\text{Grain yield} \times (100 - \text{actual grain moisture \%})}{85}$$

AQUA-BOY, KPM (moister tester) was used to determine moisture level of each sample.

Statistical analysis

Combined ANOVA was performed across all research conditions

(drought and well watered) for DT 50%, DS 50%, ASI, leaf rolling, leaf senescence, PASP, PHT, EHT, EPP, ear weight, 100 grain weight and GY per hectare using a software Plant Breeding Tools version 1.4 of June 2014 (Griffing's 1956) method 1 model 1. Mean squares and error were also computed.

The GCA effects of inbreds and SCA effects of the hybrids as well as their mean squares in each environment, were estimated on the 5 × 5 diallel mating design, excluding the checks, following Griffing's method 1 model 1 (fixed model), Griffing (1956) (Table 1) by using a software Plant Breeding Tools version 1.4 of June 2014. Standard errors for each parent and cross as well as LSD were computed. The program also computed genetic variance components (V_A and V_D).

Also, GCA effects of inbreds and SCA effects of the hybrids as well as their mean squares were performed using Diallel SAS program developed by Zhang et al. (2005) modified in 2009 adopted to SAS software version 9.0. Means and coefficient of variation (CV) of all genotypes (parents, crosses and checks) were performed using GenStat (2009). High-parent heterosis (HPH) and mid-parent heterosis (MPH) were estimated according to the formulae given by Fehr (1987) as follow:

$$\text{High-Parent Heterosis (\%)} = \frac{F_1 - HP}{HP} \times 100$$

$$\text{Mid-Parent Heterosis (\%)} = \frac{F_1 - MP}{MP} \times 100$$

where HPH = High-Parent Heterosis, MPH = Mid-Parent Heterosis, F_1 = Performance of hybrid, HP = Performance of best parent, and MP = Average performance of parents per se (parent 1 + parent 2)/2

Also, the estimate of genetic variability parameters and heritability were computed as follow:

(i) Genotypic and phenotypic variance as well as heritability in broad sense were computed using the formulae given by Singh and Chaudhary (1985) in case of one location.

$$\text{Broad sense heritability (h}^2_b\text{)} = \sigma^2_g / \sigma^2_{ph} \times 100$$

For individual location, variance components were computed from mean squares and expected mean squares as follow:

$$\begin{aligned} \sigma^2_g &= (MS_g - MS_e)/r \\ \sigma^2_e &= MS_e/r \\ \sigma^2_{ph} &= \sigma^2_g + \sigma^2_e \end{aligned}$$

where MS_g = mean squares due to genotypes, σ^2_g = genotypic variance, MS_e = error mean square, σ^2_e = error variance, r = number of replications, σ^2_{ph} = phenotypic variance.

(ii) Heritability in narrow sense was calculated by the formulae given by Grafius et al. (1952).

$$\text{Narrow sense heritability (h}^2_n\text{)} = (\sigma^2_f + \sigma^2_m) / (\sigma^2_f + \sigma^2_m + \sigma^2_{fm} + \sigma^2_e/r) \times 100$$

where σ^2_f = genetic variance of female, σ^2_e = error variance, σ^2_m = genetic variance of male r = number of replication, σ^2_{fm} = genetic variance of females × males.

The heritability values were classified as low (<30%), moderate (30-60%) and high (>60%) according to Johnson et al. (1955). According to Singh and Chaudhary (1985), the estimates of variances due to GCA_f , GCA_m and SCA in one location were

Table 1. Combined analysis of variance and variance component of twelve maize characters in full diallel cross under drought and well watered conditions.

Source of variance	DF	DT 50%	DS 50%	ASI	Leaf R	Leaf S	PASP	PHT	EHT	EPP	Ear W	100 GW	GY
Entry	24	63.2**	51.7**	20.3 ^{ns}	0.4 ^{ns}	8.1 ^{ns}	3.0*	3867.9**	1089.9**	0.008 ^{ns}	1728.7 ^{ns}	7.7 ^{ns}	2419936*
Variance component													
GCA	4	236.5**	217.7*	57.3 ^{ns}	0.11 ^{ns}	22.3 ^{ns}	4.0 ^{ns}	14571.1*	3587.2**	0.007 ^{ns}	2494.6 ^{ns}	13.3 ^{ns}	2775114 ^{ns}
SCA	10	40.8**	23.4 ^{ns}	22.1 ^{ns}	0.69 ^{ns}	4.4 ^{ns}	5.0*	2465.1**	949.7**	0.0099**	2863.4 ^{ns}	8.6 ^{ns}	4292141 ^{ns}
Reciprocal	10	16.3**	13.6 ^{ns}	3.7 ^{ns}	0.21 ^{ns}	6.1 ^{ns}	1.0 ^{ns}	989.5**	231.2**	0.0067 ^{ns}	287.7 ^{ns}	4.7 ^{ns}	405660 ^{ns}
GCA/SCA		5.80	9.30	2.60	0.16	5.00	0.80	5.90	3.80	0.700	0.87	1.55	0.65
Entry × Env	24	4.1 ^{ns}	11.6**	10.2**	0.64**	7.7**	1.2**	272.1 ^{ns}	64.5 ^{ns}	0.0048 ^{ns}	888.5**	3.9 ^{ns}	1125568**
GCA × Env	4	14.6**	26.9**	12.3**	0.70*	20.9 ^{ns}	2.5**	1117.9**	85.1 ^{ns}	0.0025 ^{ns}	1282.2**	2.4 ^{ns}	1236991**
SCA × Env	10	1.8 ^{ns}	10.3**	17.2**	10.7**	4.0 ^{ns}	1.4**	143.9 ^{ns}	88.2 ^{ns}	0.002 ^{ns}	1310.1**	6.8**	1601067**
Rec × Env	10	4.2 ^{ns}	6.8*	2.5**	0.19 ^{ns}	6.2 ^{ns}	0.6**	61.8 ^{ns}	32.6 ^{ns}	0.0086 ^{ns}	309.4**	1.7 ^{ns}	605499**
Residual	100	2.8	3.3	0.7	0.23	0.3	0.2	206.0	62.7	0.0068	120.2	2.6	128504

DT 50%: Day 50% tasseling, DS 50%: days 50% silking, ASI: anthesis-silking interval, Leaf R: leaf rolling, Leaf S: leaf senescence, PASP: plant aspect, PHT: plant height, EHT: ear height, EPP: ears per plant, Ear W: ear weight, 100 GW: 100 grain weight, GY: grain yield per hectare, Env: environment, Rec: reciprocal, GCA: general combining ability, SCA: specific combining ability, ns: non-significant, *Significant at 0.05 probability level, **Highly significant at 0.01% probability level.

computed from mean squares as follow:

$$\text{COV(H.S)}_f = \sigma^2_f = (\text{MS}_f - \text{MS}_{fm})/rm$$

$$\text{COV(H.S)}_m = \sigma^2_m = (\text{MS}_m - \text{MS}_{fm})/rf$$

$$\text{COV(F.S)} = (\text{MS}_{fm} - \text{MS}_e)/r$$

where MS_f = mean square of female, f = number of females, MS_m = mean square of male, m = number of males, MS_e = mean square of error, r = number of replications, MS_{fm} = mean square of females × males.

RESULTS

The results of combined analysis of variance (ANOVA) for twelve maize traits studied under drought stress and well watered conditions are presented in Table 1. Mean squares for environments were highly significant ($p < 0.01$) for all the traits except DT 50% and EEP, while only DT 50%, DS 50%, PHT and EHT were highly significant ($p < 0.01$) for entries. The interaction

effects of entry × environment, GCA × environment and SCA × environment were highly significant ($p < 0.01$) for all the traits studied except DT 50%, leaf senescence, PHT, EHT, EPP and 100 seed weight. On the contrary, reciprocal × environment interaction effect was highly significant ($p < 0.01$) for only ASI, PASP, ear weight and GY and significant ($p < 0.05$) for DS 50%. Partitioning the entry mean squares into components showed that GCA mean squares was highly significant ($p < 0.01$) for only DT 50% and EHT and significantly different ($p < 0.05$) for DS 50% and PHT. Specific combining ability was significantly different ($p < 0.01$) for DT 50%, PHT, EHT and EPP and significant ($p < 0.05$) for PASP. All the characters showed more than unity GCA/SCA ratio values except leaf rolling, plant aspect, EPP, ear weight and GY. The results of GCA effects of inbreds in full diallel cross for eight traits under two conditions are

presented in Table 2. Entry TZEI-23 exhibited highly significant ($p < 0.01$) negative GCA (highest negative for DT 50% and DS 50%) effects under drought stress and well watered conditions for DT 50% (drought -4.02 and well watered -2.94) and DS 50% (drought -3.63 and well watered -2.07). In addition, this entry recorded also the highest highly significant ($p < 0.01$) positive GCA effects for ear weight (3.47) and GY (215.22) under drought condition. Therefore in terms of GCA, for a set of the aforementioned traits, TZEI-23 comes out successful in general. On the contrary, TZEI-124 showed highly significant ($p < 0.01$) positive GCA effect under water stress and well watered conditions for DT 50%, DS 50%, ASI, PHT and leaf senescence. Thus TZEI-124 was the lowest GCA effect in combination of these traits. TZEI-25 had the highest highly ($p < 0.01$) negative general combining ability effect for ASI (-1.64) under drought condition. TZEI-13 recorded the highly

Table 2. General combining ability effects of eight maize characters in full diallel cross under drought and well watered conditions.

Inbreds	DT 50%		DS 50%		ASI		Leaf S		PASP		PHT		Ear W		GY	
	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW
TZEI-13	1.31**	1.39**	2.31**	1.79**	1.43**	1.07**	0.74**	0.01 ^{ns}	0.31**	0.27**	-10.59**	-18.26**	-4.74**	-12.87**	-117.82**	-443.98**
TZEI-17	0.11 ^{ns}	0.83**	-0.56 ^{ns}	0.66*	-0.54**	-0.67**	-1.82**	-0.02 ^{ns}	-0.11 ^{ns}	0.30**	-9.18**	-12.23**	-0.29 ^{ns}	-8.77**	-83.09**	-254.70**
TZEI-23	-4.02**	-2.94**	-3.63**	-2.07**	-0.47**	-0.83**	0.14 ^{ns}	-0.01 ^{ns}	-0.06 ^{ns}	0.29**	-4.34*	-11.98**	3.47**	0.74 ^{ns}	215.22**	110.40 ^{ns}
TZEI-25	1.88**	0.46 ^{ns}	0.21*	-1.41**	-1.64**	-0.07 ^{ns}	-0.37**	-0.01 ^{ns}	-0.05 ^{ns}	-0.18*	7.70**	18.06**	2.44**	9.96**	76.84**	350.77**
TZEI-124	0.71**	0.26 ^{ns}	1.67**	1.03**	1.23**	0.50**	1.31**	0.03*	-0.09 ^{ns}	-0.68**	16.4**	24.41**	-0.88 ^{ns}	10.95**	-91.13**	237.51**
SE	3.89	2.06	3.83	1.97	1.29	0.45	1.02	-	0.03	0.15	96.73	274.93	8.81	98.13	16398.84	110329

DT 50%: Day 50% tasseling, DS 50%: days 50% silking, ASI: anthesis-silking interval, Leaf R: leaf rolling, Leaf S: leaf senescence, PASP: plant aspect, PHT: plant height, EHT: ear height, GY: grain yield per hectare, DS: drought stress, WW: well watered; ns: non-significant, *Significant at 0.05 probability level, **Highly significant at 0.01% probability level, SE: standard error.

negative significant ($p < 0.01$) GCA for PHT (Table 2). Considering the lines individually for each trait under the two conditions TZEI-13 was the best donor for plant height. In terms of grain yield and its component, TZEI-23 had the highest and highly positive ($p < 0.01$) GCA under drought condition for ear weight (3.47) and grain yield per hectare (215.22) while under well watered condition TZEI-25 showed the highly positive GCA for grain yield (350.77). TZEI-25 exhibited highly significant positive GCA under both conditions for ear weight (2.44 and 9.96) and grain yield (76.84 and 350.77). Therefore, TZEI-23 was the best general donor for ear weight and GY under drought while TZEI-25 was the best general donor for ear weight and GY under well watered condition (Table 2).

The estimates of SCA of eight characters of twenty hybrids (set of F_1 s and reciprocal F_1) under drought stress and well watered conditions are presented in Table 3. Hybrids TZEI-25 \times TZEI-13 showed the highest positive and highly significant ($p < 0.01$) SCA effects for GY (385.74) followed by its reciprocal TZEI-13 \times TZEI-25 (311.49) under water stress while under well watered condition, TZEI-13 \times TZEI-124 (1132.01),

TZEI-17 \times TZEI-13 (789.01) and TZEI-17 \times TZEI-124 (789.01) were the highest and were highly significant ($p < 0.01$) (Table 3). Cross TZEI-17 \times TZEI-25 was the only one that exhibited best performance and showed highly positive and significant ($p < 0.01$) SCA across both environments (218.76 under drought and 655.38 under well watered condition) for GY. For the reproductive traits, only entry TZEI-17 \times TZEI-13 exhibited the highest and highly significant ($p < 0.01$) negative SCA for DT 50%, DS 50% and ASI. However, TZEI-23 \times TZEI-124 was the best in terms of SCA effect for DT 50% and DS 50% under water stress and well watered conditions, respectively. For ASI only TZEI-13 \times TZEI-25 (-0.73), TZEI-13 \times TZEI-124 (-1.13) and TZEI-17 \times TZEI-13 (-1.67) showed highly negative significant SCA and TZEI-124 \times TZEI-17 (-0.67) showed negative and significant ($p < 0.05$) under well watered condition (Table 3). For leaf senescence under drought, five crosses showed highly negative significant ($p < 0.01$) ranged from -0.78 (TZEI-23 \times TZEI-25) to -3.56 (TZEI-25 \times TZEI-13), whereas one cross TZEI-25 \times TZEI-23 (-0.78) exhibited negative significant ($p < 0.05$) SCA. For PHT, either under drought or normal conditions,

only two hybrids TZEI-17 \times TZEI-124 (-1.94) and TZEI-23 \times TZEI-124 (-1.57) showed negative significant ($p < 0.05$) SCA.

Results from both narrow sense and broad sense heritability estimates in this study under drought stress and well watered conditions are presented in Table 4. Environment played its role in modifying narrow sense heritability while heritability in broad sense was not much influenced by environment. The heritability values were classified as low (<30%), moderate (30-60%) and high (>60%) according to Johnson et al. (1955). The estimates of narrow sense heritability were high for only DT 50% (0.69), DS 50% (0.80) and leaf senescence (0.61) under drought while for plant height (0.65) under well watered condition (Table 4). It was moderate (0.50) for DT 50% under well watered. High magnitudes of broad sense heritability were found in all characters under both water stress and well watered conditions except for leaf senescence (0.12) under well watered condition and hundred grain weight under both conditions (0.56 each). The following characters had high heritability above greater or equal to 0.90% under drought stress; DT 50% (0.94), DS 50% (0.90), ASI (0.96),

Table 3. Specific combining ability effects of eight maize characters in full diallel under drought and well watered conditions.

Crosses	DT 50%		DS 50%		ASI		Leaf S		PASP		PHT		Ear W		GY	
	DS	WW	DS	WW												
TZEI-13 × TZEI-17	0.52 ^{ns}	1.57*	1.79**	0.97 ^{ns}	0.97**	0.87**	-0.86**	-0.01 ^{ns}	0.37*	0.34*	-5.96 ^{ns}	-6.21 ^{ns}	-4.98**	-14.13**	-271.14**	-507.37**
TZEI-13 × TZEI-23	-0.68 ^{ns}	-0.83 ^{ns}	0.03 ^{ns}	-0.46 ^{ns}	-0.59 ^{ns}	0.87**	0.51*	0.03 ^{ns}	-0.08 ^{ns}	-0.81**	2.53 ^{ns}	10.27 ^{ns}	3.77**	11.72*	121.35*	469.84**
TZEI-13 × TZEI-25	0.25 ^{ns}	-0.39 ^{ns}	-0.47 ^{ns}	-0.29 ^{ns}	1.74**	-0.73**	1.19**	0.03 ^{ns}	-0.10 ^{ns}	-0.40*	10.83*	13.22*	5.01**	9.53*	311.49**	242.06 ^{ns}
TZEI-13 × TZEI-124	-1.75**	-1.53*	0.11 ^{ns}	-3.06**	0.71**	-1.13**	-0.69 ^{ns}	0.03 ^{ns}	0.07 ^{ns}	-0.40*	6.21 ^{ns}	10.33*	-2.62 ^{ns}	30.44**	76.38*	1132.01**
TZEI-17 × TZEI-13	-3.00**	-3.50**	-1.50 ^{ns}	-4.00**	-0.17 ^{ns}	-1.67**	0.06 ^{ns}	0.00 ^{ns}	-0.08 ^{ns}	-0.78**	24.28**	23.33**	-2.46 ^{ns}	19.33**	-69.94 ^{ns}	789.01**
TZEI-17 × TZEI-23	-0.98 ^{ns}	-1.76**	-0.61 ^{ns}	-0.49 ^{ns}	0.87*	0.43 ^{ns}	-0.32 ^{ns}	0.01 ^{ns}	-0.12 ^{ns}	-0.18*	4.40 ^{ns}	7.90 ^{ns}	2.55 ^{ns}	8.46 ^{ns}	219.42**	434.11*
TZEI-17 × TZEI-25	-0.88 ^{ns}	-0.83 ^{ns}	-1.77**	-0.83 ^{ns}	1.37**	0.33 ^{ns}	0.25 ^{ns}	0.01 ^{ns}	-0.65**	-0.82**	12.04**	20.97**	7.91**	17.80**	218.76**	655.38**
TZEI-17 × TZEI-124	-0.38 ^{ns}	-0.96 ^{ns}	0.09 ^{ns}	-1.43*	-0.33 ^{ns}	0.10 ^{ns}	-0.49 ^{ns}	-0.06 ^{ns}	-0.20 ^{ns}	-0.21*	-2.39 ^{ns}	-1.21 ^{ns}	2.67*	24.08**	37.15 ^{ns}	789.01**
TZEI-23 × TZEI-13	0.33 ^{ns}	-1.00 ^{ns}	-0.33 ^{ns}	-0.50 ^{ns}	-0.67 ^{ns}	0.17 ^{ns}	-1.39**	-0.06 ^{ns}	-0.19 ^{ns}	-0.61**	2.50 ^{ns}	-0.17 ^{ns}	3.36 ^{ns}	5.67 ^{ns}	155.75*	159.23 ^{ns}
TZEI-23 × TZEI-17	-0.17 ^{ns}	0.17 ^{ns}	-0.50 ^{ns}	2.67**	0.17 ^{ns}	1.67**	0.00 ^{ns}	0.00 ^{ns}	-0.33 ^{ns}	0.06 ^{ns}	-3.89 ^{ns}	-3.39 ^{ns}	-1.09 ^{ns}	-0.03 ^{ns}	-24.96 ^{ns}	274.60 ^{ns}
TZEI-23 × TZEI-25	-1.08*	-0.73 ^{ns}	-1.37*	-0.59 ^{ns}	1.47**	0.50*	-0.78**	0.00 ^{ns}	0.21 ^{ns}	-0.59**	7.14 ^{ns}	5.39 ^{ns}	2.41 ^{ns}	0.55 ^{ns}	39.67 ^{ns}	61.11 ^{ns}
TZEI-23 × TZEI-124	-1.25*	-1.53*	-1.01*	-1.03*	-0.39 ^{ns}	0.27 ^{ns}	-0.21 ^{ns}	-0.05 ^{ns}	-0.39*	-0.31*	0.37 ^{ns}	0.49 ^{ns}	4.18**	0.82 ^{ns}	268.03**	235.94 ^{ns}
TZEI-25 × TZEI-13	-0.50 ^{ns}	-0.17 ^{ns}	-0.33 ^{ns}	0.00 ^{ns}	-0.17 ^{ns}	0.33 ^{ns}	-3.56**	0.06 ^{ns}	-0.22 ^{ns}	-0.11 ^{ns}	4.28 ^{ns}	3.07 ^{ns}	3.72*	0.17 ^{ns}	385.74**	-110.32 ^{ns}
TZEI-25 × TZEI-17	1.83**	0.50 ^{ns}	1.83*	1.00 ^{ns}	0.83*	0.33 ^{ns}	-0.28 ^{ns}	0.00 ^{ns}	-0.14 ^{ns}	0.05 ^{ns}	3.79 ^{ns}	3.28 ^{ns}	6.84**	1.78 ^{ns}	92.97 ^{ns}	-107.56 ^{ns}
TZEI-25 × TZEI-23	0.50 ^{ns}	1.17 ^{ns}	0.17 ^{ns}	0.50 ^{ns}	-0.33 ^{ns}	-0.33 ^{ns}	-0.78*	0.00 ^{ns}	-0.28 ^{ns}	0.17 ^{ns}	6.39 ^{ns}	13.5*	-2.55 ^{ns}	1.42 ^{ns}	-30.65 ^{ns}	125.83 ^{ns}
TZEI-25 × TZEI-124	-1.15*	0.24 ^{ns}	0.66 ^{ns}	0.81 ^{ns}	2.77**	0.17 ^{ns}	-1.95**	-0.05 ^{ns}	-0.37*	-0.34*	5.06 ^{ns}	5.45 ^{ns}	5.38**	5.73 ^{ns}	64.81 ^{ns}	129.57 ^{ns}
TZEI-124 × TZEI-13	-0.67 ^{ns}	0.50 ^{ns}	0.83 ^{ns}	-0.33 ^{ns}	1.00*	0.17 ^{ns}	-0.42 ^{ns}	-0.06 ^{ns}	0.14 ^{ns}	0.28 ^{ns}	4.25 ^{ns}	6.72 ^{ns}	-3.23 ^{ns}	-10.9 ^{ns}	151.34*	-389.41 ^{ns}
TZEI-124 × TZEI-17	0.50 ^{ns}	0.83 ^{ns}	-0.83 ^{ns}	0.17 ^{ns}	-0.67 ^{ns}	-0.67*	1.00**	0.00 ^{ns}	-0.22 ^{ns}	0.28 ^{ns}	11.72*	3.22 ^{ns}	2.44 ^{ns}	-14.91*	35.06 ^{ns}	-510.65*
TZEI-124 × TZEI-23	0.83 ^{ns}	-0.17 ^{ns}	1.00 ^{ns}	-0.50 ^{ns}	-0.33 ^{ns}	-0.33 ^{ns}	1.67**	0.00 ^{ns}	0.36 ^{ns}	-0.39 ^{ns}	-3.89 ^{ns}	2.61 ^{ns}	-1.56 ^{ns}	10.08 ^{ns}	-95.27 ^{ns}	443.10*
TZEI-124 × TZEI-25	-0.17 ^{ns}	-0.67 ^{ns}	0.17 ^{ns}	-0.67 ^{ns}	-0.67 ^{ns}	0.67*	1.11**	0.00 ^{ns}	0.33 ^{ns}	0.00 ^{ns}	-8.17 ^{ns}	-3.17 ^{ns}	3.88*	4.69 ^{ns}	215.31**	214.39 ^{ns}
SE SCA	1.85	1.93	1.07	1.92	3.07	0.42	0.74	na	0.11	0.45	71.13	160.96	30.72	339.72	49340.76	473679.9
SE REC	0.65	0.74	0.4	1.14	0.15	0.31	0.91	na	0.03	0.06	41.5	38.19	5.37	39.86	11999.42	63868.44

DT 50%: Day 50% tasseling, DS 50%: days 50% silking, ASI: anthesis-silking interval, Leaf R: leaf rolling, Leaf S: leaf senescence, PASP: plant aspect, PHT: plant height, EHT: ear height, GY: grain yield per hectare, DS: drought stress, WW: well watered; SE: standard error, SCA: specific combining ability, REC: reciprocal, na: Not available, ns: non-significant, *Significant at 0.05 probability level, **Highly significant at 0.01% probability level.

leaf senescence (0.96), EHT (0.90), ear weight (0.92) and GY (0.94) while under well watered ASI (0.94), PASP (0.93), PHT (0.91), ear weight (0.91) and GY (0.93) had broad sense heritability greater or equal to 0.90%.

The estimates of high parent heterosis (HPH) were computed for GY and yield related traits under water stress and well watered conditions as

presented in Table 5. The results revealed that hybrids TZEI-25 × TZEI-17 (-5.93% and -5.87% for DT 50% and -6.49% and -1.42% for DS 50%) and TZEI-124 × TZEI-13 (-7.04% and -9.30% for DT 50% and -0.60 and -11.60 for DS 50%) showed negative HPH for days to 50% tasseling and silking under both water stress and well watered conditions. However, hybrids TZEI-13 ×

TZEI-124 (-9.85%) and TZEI-124 × TZEI-17 (-9.35%) showed the highest negative high parent heterosis for DT 50% under drought stress and well watered conditions, respectively and TZEI-25 × TZEI-17 (-6.49%) and TZEI-13 × TZEI-124 (-12.80%) performed also the highest high parents heterosis for DS 50% under drought stress and well watered conditions, respectively. Regarding

Table 4. Estimates of narrow and broad sense heritability of ten maize characters in full diallel cross under drought and well watered conditions.

Parameter	Days to 50% tasseling		Days to 50% silking		Anthesis-silking interval		Leaf senescence		Plant aspect	
	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW
σ^2_g	12.43	8.13	10.25	8.61	7.55	2.14	5.08	0.0004	0.20	1.04
σ^2_{ph}	13.25	9.19	11.39	9.70	7.89	2.29	5.26	0.003	0.28	1.13
σ^2_e	0.81	1.05	1.38	1.09	0.33	0.15	0.19	0.003	0.07	0.08
h^2_n	0.69	0.50	0.80	0.54	0.05	0.43	0.61	0.01	0.03	0.03
h^2_b	0.94	0.89	0.90	0.89	0.96	0.94	0.96	0.12	0.73	0.93

	Plant height		Ear height		Ear weight		Hundred grain weight		Grain yield per hectare	
	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW
σ^2_g	365.29	877.34	148.43	194.94	69.6	722.63	0.99	1.20	125445.2	1170124
σ^2_{ph}	414.19	965.81	164.76	220.05	75.27	797.13	1.76	2.13	133690.71	1286559.6
σ^2_e	48.90	88.47	16.67	25.11	5.66	74.50	0.77	0.93	8245.51	116435.6
h^2_n	0.52	0.65	0.36	0.43	0.13	0.12	0.05	0.20	0.001	0.25
h^2_b	0.88	0.91	0.90	0.89	0.92	0.91	0.56	0.56	0.94	0.93

DS: Drought stress, WW: well watered, σ^2_g : genotypic variance, σ^2_{ph} : phenotypic variance, σ^2_e : error variance, h^2_n : narrow sense heritability, h^2_b : broad sense heritability.

Table 5. Heterosis percentages (%) over better parents for six traits in full diallel cross under drought and optimal conditions.

Crosses	DT 50%		DS 50%		ASI		PHT		Ear W		GY	
	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW
TZEI-13 x TZEI-17	-6.67	-7.19	5.20	-7.13	38.57	-200.00	20.77	24.19	-29.02	180.98	-41.54	221.97
TZEI-13 x TZEI-23	2.56	-5.62	5.99	0.71	0.00	-250.00	10.68	21.06	75.38	46.67	104.77	81.78
TZEI-13 x TZEI-25	-2.81	-2.93	-3.00	4.32	30.95	75.19	25.07	14.57	155.74	33.76	364.48	19.74
TZEI-13 x TZEI-124	-9.85	-7.16	2.42	-12.80	100.23	-30.03	-4.27	-4.92	-7.38	161.35	158.79	322.47
TZEI-17 x TZEI-13	6.67	7.92	10.05	8.46	1818.18	-451.13	-19.55	-10.49	9.06	-6.6	-7.72	-17.38
TZEI-17 x TZEI-23	-2.56	-6.41	-2.10	4.99	1212.12	-216.50	8.17	19.49	67.67	37.73	96.3	99.05
TZEI-17 x TZEI-25	2.22	-3.66	0.66	2.89	1212.12	-225.56	26.73	56.47	198.37	56.82	163.38	47.85
TZEI-17 x TZEI-124	-2.22	-5.74	1.95	-5.18	1212.12	-175.19	-4.11	-9.13	97.99	143.54	64.85	284.72
TZEI-23 x TZEI-13	0.85	-0.82	6.98	2.85	166.50	-233.50	6.64	21.32	32.59	25.77	49.76	61.37
TZEI-23 x TZEI-17	-1.72	-7.20	0.00	-6.43	100.00	-50.00	14.45	24.52	81.51	37.82	105.16	63.81
TZEI-23 x TZEI-25	3.41	-2.40	-0.71	-3.58	66.50	-150.00	28.74	19.75	74.81	43.46	91.82	48.06
TZEI-23 x TZEI-124	0.85	-8.02	4.89	-2.14	116.50	-166.50	-8.8	-8.51	71.3	78.57	91.04	128.71
TZEI-25 x TZEI-13	-0.7	-2.21	-1.81	4.32	-194.79	25.56	18.35	10.99	87.89	33.22	115.63	29.97
TZEI-25 x TZEI-17	-5.93	-5.87	-6.49	-1.42	-135.86	-175.19	20.79	18.91	92.41	50.94	118.38	57.85

Table 5. Contd.

TZEI-25 × TZEI-23	0.85	-8.02	-1.40	-5.74	-163.19	-183.50	18.69	4.01	107.33	38.77	102.63	36.37
TZEI-25 × TZEI-124	-9.53	-5.14	-1.19	3.60	-174.79	125.56	-1.5	5.13	215.45	74.22	246.55	61.25
TZEI-124 × TZEI-13	-7.04	-9.30	-0.60	-11.60	66.75	-39.94	-9.25	-11.2	51.46	223.27	61.28	429.5
TZEI-124 × TZEI-17	-4.44	-9.35	5.20	-5.84	33.25	-275.19	-17.86	-12.14	60.22	228.28	47.94	425.04
TZEI-124 × TZEI-23	-3.41	-7.20	0.69	0.00	25.00	-200.00	-4.24	-10.94	91.14	41.45	124.73	71.74
TZEI-124 × TZEI-25	-8.84	-2.21	-1.81	6.48	83.25	25.56	8.08	8.09	140.14	58.75	104.36	41.33

DT 50%: Day 50% tasseling, DS 50%: days 50% silking, ASI: anthesis-silking interval, PHT: plant height, Ear W: ear weight, GY: grain yield per hectare, DS: drought stress, WW: well watered; ns = Not available, ns = non-significant.

these results, hybrids TZEI-25 × TZEI-17 and TZEI-124 × TZEI-13 came out as the best for days to DT 50% and DS 50% under both water stress and well watered conditions. For ASI, four hybrids with line TZEI-25 as female parent exhibited negative high parents heterosis under drought but 16 under well watered. The inbred TZEI-25 gave maximum heterosis under well watered when used either female or male parent, it performed well under drought when used as female. Eight and seven hybrids expressed negative high parent heterosis under both water stress and well watered conditions for plant height with TZEI-17 × TZEI-13 (-19.55 and -10.49%) and TZEI-124 × TZEI-17 (-17.86 and -12.14%) having the highest negative high parent heterosis. The lines TZEI-17 gave maximum heterosis in various cross combination when used as either female or male. Eighteen hybrids had positive HPH under water stress and ranged from 47.94% (TZEI-124 × TZEI-17) to 364.48% (TZEI-13 × TZEI-25) while under well watered condition 19 hybrids had positive HPH and ranged from 19.74% (TZEI-13 × TZEI-25) to 429.50% (TZEI-124 × TZEI-13) for GY. For ear weight 18 and 19 hybrids under water stress and well watered conditions, respectively, exhibited positive HPH. The values ranged from 9.06% (TZEI-17 × TZEI-13) to 215.45% (TZEI-25 × TZEI-124) under drought condition and from

25.77% (TZEI-23 × TZEI-13) to 228.28% (TZEI-124 × TZEI-17) under well watered condition. All the heterosis percentage above 100% under drought for ear weight were obtained when TZEI-25 was used either as female or male parent, whereas under normal condition TZEI-13, TZEI-17 and TZEI-124 were used as parents, hence under water stress TZEI-25 gave the maximum heterosis. TZEI-13 × TZEI-25 (364.48%) and TZEI-25 × TZEI-124 (246.55%) expressed the highest higher positive heterosis for GY under drought stress and TZEI-124 × TZEI-13 (429.50%) and TZEI-124 × TZEI-17 (425.04%) under well watered condition. TZEI-25 gave the maximum heterosis under drought condition when used either female or male parents while TZEI-124 gave the maximum under well watered condition when used as female.

DISCUSSION

Highly significant ($p < 0.01$) environment mean squares for all the traits in the current study was observed except DT 50% and EPP. Similar results were published from other studies, such as grain yield (Doerksen et al., 2003; Laouali, 2014), DS 50% (Zare et al., 2011), leaf senescence (Badu-Apraku, 2011), plant aspect (Laouali, 2014) and

ASI, PHT, EHT, leaf rolling (Premlatha and Kalamani, 2010; Aminu and Izge, 2013; Aminu et al., 2014a; Murtadha et al., 2016). This indicated that these traits were highly influenced by environmental factors and there is adequate genetic variability among the inbred lines to allow good progress from selection for improvement in the traits (Badu-Apraku et al., 2011). The significance of the entry in DT 50%, DS 50%, PHT, EHT and GY indicated that there was possibility for the improvement of these traits through selection (Badu-Apraku et al., 2011). Significant ($p < 0.05$) and highly significant ($p < 0.01$) GCA × environment, SCA × environment, reciprocal × environment and entry × environment mean squares for the following characters; DS 50%, ASI, PHT, ear weight and GY indicate that there is a significant variation in the combining ability of the inbred under different environmental conditions (Badu-Apraku et al., 2005, 2007, 2011). This exhibited that the potential performance of the inbreds and the F1 hybrids was affected by the frequency and the amount of water applied to each condition. Thus testing inbred lines (parents) under different environmental conditions will ensure selection of stable parents that can perform the potential of that environment (Machado et al., 2009; Murtadha et al., 2016) or interested in the influence of

environment in phenotypic expression of traits (Bello and Olaoye, 2009; Murtadha et al., 2016). The predominance of GCA mean square over SCA mean square indicates that additive genetic action was more important than non-additive genetic action. The results of seven traits corroborate the findings of Sharma et al. (2004) and Aminu and Izge (2013) who found the predominance of additive genetic effects in maize traits control. However, these results are in disagreement with the findings of other researchers such as Abdel-Moneam et al. (2009), Machado et al. (2009), Aminu et al. (2014a, b) and Murtadha et al. (2016) who reported the predominance of non-additive gene effects for DT 50%, DS 50%, ASI, leaf rolling, PHT, EHT and GY. For grain yield, results found in this study showed the predominance of SCA mean square over GCA mean square which indicates that non-additive genetic action was more important than additive genetic action. However, it contradicts finding of Ojo et al. (2007) who found the preponderance of additive genetic action for grain yield.

The estimates of GCA effects of five parents used in this study revealed that none of the parents had good GCA for all the traits either under one or both environmental conditions, thus the exhibition of variation both in direction and magnitude. However, comparing the parents with each other, high significance negative GCA observed by TZEI-23 for DT 50% and DS 50% and by TZEI-13 for PHT, can rank TZEI-23 as the best combiner in breeding program for early maturity and TZEI-13 for resistance to drought. Besides inbred lines with high negative GCA effects for DT 50%, DS 50% and PHT are desirable for selection under drought environment as these parents could escape drought. Similar results were reported by Izge et al. (2007), Aminu and Izge (2013) and Aminu et al. (2014a). Furthermore, TZEI-23 and TZEI-25 were good donors for ear weight and grain yield. This suggests that inbreds possess high frequency of favorable genes for selection of grain. Similar result has been reported by Haydar and Paul (2014). In addition, parents which showed good GCA for at least one trait can be as good donor parents for the accumulation of favorable genes. This result corroborated the findings of Khalil et al. (2010), Singh et al. (2012), and Haydar and Paul (2014). The worst general combiner for both reproductive traits and grain yield was TZEI-17 which did not exhibit even one best performance in any trait either under drought or well watered condition. The general best combiner was ranked as followed: TZEI-23 > TZEI-25 > TZEI-124 > TZEI-13 > TZEI-17.

The high estimates of SCA for GY for TZEI-17 × TZEI-25, TZEI-25 × TZEI-13 and TZEI-13 × TZEI-124 under water stress and well watered conditions suggest these hybrids as good combiners and their selection would lead to improvement in these characters. However, hybrid TZEI-13 × TZEI-17 which showed higher negative SCA effects for GY indicates the unsuitability of both parents as good specific combiners for grain yield. This report

supported the findings of Pswarayi and Vivek (2008) and Murtadha et al. (2016) who also observed differences in the expression of GCA and SCA with stress. Under both conditions, TZEI-17 × TZEI-25 was the most promising cross for improving GY followed by TZEI-13 × TZEI-23 due to the highest positive SCA on one hand and high significant SCA on other hand for the following characters GY and ear weight. In addition, in this study, the highest SCA effects for GY were obtained from high × low and low × high combiners in the crosses TZEI-25 × TZEI-13 and TZEI-13 × TZEI-25 under drought condition. However, low × high were observed in TZEI-13 × TZEI-124 and TZEI-17 × TZEI-124 under well watered condition. Results are in agreement with those obtained from Alam et al. (2008), Singh et al. (2012) and Haydar and Paul (2014) who reported that the superiority of high × low or average × low could be explained on the basis of interaction between positive alleles from good/average combiners and negative alleles from the poor combiners as parents. The high yield of such hybrids would be non-fixable and thus could be exploited for heterosis breeding. Some of the hybrids were obtained from low × low general combiners as in the case of TZEI-17 × TZEI-13. This supported the results of Premalatha and Kalamani (2010) and Aminu and Izge (2013). Hallauer and Miranda (1988) and Majid et al. (2010) reported that in low × low GCA combination, the superior cross could result from over dominance or epistasis. Such type of gene action may be exploited in cross-pollinated species like maize. For reproductive traits, parents TZEI-124 was the best combiner, meanwhile it was one of the two worst parents. The performance of this hybrid can be explained by the fact that it was the cross from parents with high GCA. Therefore most of the superior hybrids were from either one of the parents with high GCA effect or parents that are low × low general combiners and suggests that the parents with either high GCA and/or low SCA would have a higher chance of having excellent complementary genes with other parents that have high general combining ability. This supports results of Premalatha and Kalamani (2010) and Aminu and Izge (2013). Even though, some few crosses showed negative and significant SCA effect under both environments in respect of plant height, negative values of SCA in these traits mostly under drought are desirable as found in the studies of Aminu et al. (2014a) and Umar (2015) who reported that negative SCA effects in stress environments for plant and ear height are desirable especially in drought prone and windy areas against water stress and lodging.

Percentage of heritability in narrow sense greater than 50% recorded indicates that these traits were controlled by additive gene action. Only days to 50% tasseling under well watered condition exhibited 0.5 narrow sense heritability and this suggests that both additive and dominance gene action are important in influencing the expression of this trait. The relatively low narrow sense

heritability recorded in almost all the traits in both environmental conditions were less than 0.5. This indicates that the expressions of the traits are mainly controlled by dominance genes. Similar results were reported by Umar (2015) who said that the best exploitation of this type of gene action would be in F_1 hybrids implying that breeding gains can be made through selfing than cross breeding, with selection being made in later generation. In this study, the heritability reported for grain yield under drought stress was lower than that reported by Bolanos and Edmeades (1996) which was 40% under drought and 60% under well watered condition. However, Falconer and Mackay (1996) reported that the magnitudes of heritability estimates are products of the traits being measured, the population being tested and the environments within which the testing is done. Therefore, the variation observed in magnitudes here are the results of the differences in the population, environment and trait of the heritability estimates. It should therefore be understood that heritability values reported for a given character, are specific to a particular population under particular environmental condition (Hallauer and Miranda, 1981). Hence, it would be better to evaluate genotypes in different target environments. All the results of heritability in broad sense showed high magnitude and this is in line with the results of studies of independent researchers such as Kashiani et al. (2008), Wannows et al. (2010), Olakojo and Olaoye (2011) and Umar (2015). This revealed that variations were transmitted to the progeny and implied the effective selection for genetic improvement of these characters. Hence, provides better opportunities for selection of plant material regarding these traits. This is in line with the results of early workers; Kashiani et al. (2008), Wannows et al. (2010), Bello et al. (2012) and Aminu et al. (2014c). Under water stress, the decreased heritability of traits indicates the need for selection of genotypes under particular environment for rapid genetic improvement. This agrees with the findings of Bolanos and Edmeades (1996) who reported decreased heritability under drought.

The negative HPH observed in days to 50% tasseling and silking indicate that these hybrids are desirable candidates for earliness since it has been reported that maize crop is most susceptible at flowering under drought stress (Claassmen and Shaw, 1970; Grant et al., 1989). Therefore, hybrid that can tassel and produce silk early can take advantage in a drought environment due to the fact that it could escape drought. For ASI only, all the four crosses with line TZEI-25 as female exhibited the highest negative HPH under drought. High negative value for DT 50%, DS 50% and ASI are actually desirable and this can be explained as these hybrids could escape drought. This result is in line with the findings of Izge and Dugie (2011), Aminu et al. (2014c) and Umar (2015). The high level of HPH has also been recorded by Umar (2015) who reported that these crosses which featured prominently in

the expression of higher level heterosis could form an initial gene pool for further breeding program in developing high yielding varieties for cultivation in the Savannas. Negative values for HPH for PHT are desirable in breeding for drought tolerance. Therefore, selection of hybrids showed that negative value is important as it implied that these hybrids could resist lodging confirming the results of Aminu et al. (2014c). High positive HPH were obtained for grain yield per hectare and ear weight. Positive HPH is actually desirable in GY and ear weight. High heterotic values for grain yield have also been reported by Joshi et al. (2002), Ojo et al. (2007), Amanullah et al. (2011) and Aminu et al. (2014a). Therefore, these hybrids could contain genes that could be introgressed to exploit heterosis for earliness and high grain yield. Similar results were reported by Kumar et al. (1998), Joshi et al. (1998), Bello and Olaoye (2009) and Aminu et al. (2014a).

Conclusion

The study revealed that both additive and non-additive gene actions were important in controlling GY and other characters in maize, and additive gene action was more important in controlling most of the traits. The best exploitation would be in F_1 hybrids implying that breeding gains can be made through selfing than cross breeding. TZEI-23 and TZEI-25 were identified as the best general combiners respectively under drought and well watered conditions. These parents could be more useful in hybridization programs with those parents with low combining abilities. Narrow sense heritability was high, medium and low. High value of heritability indicates considerable potential for development of drought tolerance and high yielding varieties through selection of desirable traits in succeeding generations. TZEI-13 × TZEI-25 in GY, ear weight, PAST, leaf senescence, DT 50% and DS 50%, and TZEI-124 × TZEI-13 in GY, ear weight, PHT, PAST, DT 50% and DS 50% had high parent heterosis.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The financial support of this research by AGRA is gratefully acknowledged. INRAN Niger and KNUST Kumasi are also gratefully acknowledged.

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Appendix 1. List of genetic materials used in the study.

Parents		Hybrids		Checks	
Entry	Name	Entry	Name	Entry	Name
Entry 1	TZEI-13	Entry 6	TZEI-13 × TZEI-17	Entry 26	MAMABA
Entry 2	TZEI-17	Entry 7	TZEI-13 × TZEI-23	Entry 27	OMANKWA
Entry 3	TZEI-23	Entry 8	TZEI-13 × TZEI-25		
Entry 4	TZEI-25	Entry 9	TZEI-13 × TZEI-124		
Entry 5	TZEI-124	Entry 10	TZEI-17 × TZEI-13		
		Entry 11	TZEI-17 × TZEI-23		
		Entry 12	TZEI-17 × TZEI-25		
		Entry 13	TZEI-17 × TZEI-124		
		Entry 14	TZEI-23 × TZEI-13		
		Entry 15	TZEI-23 × TZEI-17		
		Entry 16	TZEI-23 × TZEI-25		
		Entry 17	TZEI-23 × TZEI-124		
		Entry 18	TZEI-25 × TZEI-13		
		Entry 19	TZEI-25 × TZEI-17		
		Entry 20	TZEI-25 × TZEI-23		
		Entry 21	TZEI-25 × TZEI-124		
		Entry 22	TZEI-124 × TZEI-13		
		Entry 23	TZEI-124 × TZEI-17		
		Entry 24	TZEI-124 × TZEI-23		
		Entry 25	TZEI-124 × TZEI-25		

Full Length Research Paper

Development of two high-yielding, consumer-acceptable apple banana hybrids (*Musa* species, AAB genome group) with resistance to *Fusarium oxysporum* f. sp. *cabense* race 1

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Received 9 January, 2018; Accepted 5 March, 2018

***Fusarium* wilt of bananas (*Musa* species) is caused by *Fusarium oxysporum* f. sp. *cabense* (Foc). Foc race 1 in particular affects dessert bananas in Uganda, causing >60% yield loss. This study was conducted to assess the performance of two new apple banana genotypes for bunch yield, resistance to Foc race 1 and consumer acceptability. The new apple banana genotypes (NAMU1 and NAMU2), along with two check cultivars, one susceptible but preferred by consumers (Sukali ndiizi) and the other resistant (Yangambi-KM5), were evaluated at the National Agricultural Research Laboratories in Uganda. Bunch yields of the two new apple bananas were higher than those of check cultivars by >50%. NAMU1 and Yangambi-KM5 showed no symptoms of Foc race 1, whereas NAMU2 showed mild symptoms on its corms. Sukali ndiizi showed severe pseudostem splitting and corm discoloration as the key symptoms of Foc race 1. The consumer acceptability of NAMU1 and NAMU2 was as high as that of Sukali ndiizi, implying that they can be perfect substitutes for the Foc race 1 susceptible Sukali ndiizi.**

Key words: Dessert banana hybrids, host plant resistance, Panama disease, sensory attributes.

INTRODUCTION

Apple banana (*Musa* species, AAB genome group), is one of the more than 300 varieties of banana grown worldwide. It is the most widely distributed dessert banana cultivar in Uganda (Gold et al., 2002). Apple

banana is locally known as Sukali ndiizi and Kabaragara in the Central and Western regions of Uganda, respectively (Nsabimana and Van Staden, 2006); in Rwanda, it is known as Kamaramasenge (Nsabimana

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and Van Staden, 2006). The cultivar is known for its small fruits with a thin peel and a slightly acidic apple-like taste of the pulp, which is its unique characteristic (Van Asten et al., 2010). It makes a major contribution to Uganda's economy, as its production is mainly by small-scale farmers who sell it for improved incomes and also eat it for nutrition. Apple banana has been commonly sold and consumed fresh, but of late, it is being processed by a number of private sector and development partners to improve shelf life and value addition (Van Asten et al., 2010). This makes it fit well in the Uganda government policy of value addition of agricultural products.

Fusarium wilt, also known as Panama disease, is the most important lethal disease of dessert bananas (Butler, 2013; Dale et al., 2017). It is a fungal disease caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) (Ploetz and Evans, 2015). Foc race 1 is the primary cause of *Fusarium* wilt disease of dessert bananas in Uganda (Karangwa et al., 2016). The disease severely affects important exotic banana cultivars, such as Gross Michel, Kayinja, Kisubi and apple banana, which are major dessert and juice-producing bananas in the country (Bettina et al., 2012). It causes an estimated yield loss of >60% in dessert bananas (Tushemereirwe et al., 2000).

The first internal symptom in diseased plants is a reddish brown discoloration of the xylem that develops in feeder roots, the initial sites of infection (Butler, 2013; Ploetz, 2015). Vascular discoloration progresses to the rhizome, where the stele joins the cortex, and ultimately proceeds up to the pseudostem. On plants that are more than four months old, the oldest leaves yellow or split longitudinally at the base. Eventually, younger leaves wilt and collapse until the entire plant canopy consists of dead or dying leaves (Ploetz, 2015). Infected rhizomes are often symptomless and effectively spread the pathogen when used as planting material (Stover, 1962). The pathogen spreads in soil, running water and farm implements, and survives for up to 30 years in the absence of banana (Stover, 1962). Because of the persistent nature of Foc in the soil and the lack of effective chemical control strategies, the development of Foc-resistant cultivars has been a priority in banana genetic improvement programs (Daniells, 2011).

Control of Foc through the deployment of dessert banana varieties similar to Sukali ndiizi but are resistant to the locally prevalent Foc race 1 has not been successful in Uganda until the present time. In this paper, the performance of two consumer-acceptable apple banana genotypes possessing resistance to Foc race 1, which were developed at the National Banana Research Programme (NBRP) in Uganda was presented and discussed. In the early 1990s, scientists at the NBRP in Uganda evaluated a wide range of banana cultivars and recommended FHIA 17, FHIA 23, Cavendish and Yangabi-KM5 as resistant dessert banana cultivars to Foc race 1 (Tushemereirwe et al., 2000). Although these varieties were high-yielding and resistant to Foc race 1,

their sensory/organoleptic traits were not appealing to consumers accustomed to the taste of apple bananas (Tushemereirwe et al., 2000; Van Asten et al., 2010). Against this background, there was a need to develop new varieties of apple banana that combine resistance to Foc race 1 and desired fruit quality traits to sustainably exploit the potential of local and export markets for the apple banana. The objective of this study was to compare two newly developed consumer-acceptable apple banana genotypes with the existing commercial cultivar (Sukali ndiizi) for bunch yield, resistance to Foc race 1 and consumer acceptability.

MATERIALS AND METHODS

Experimental site

The experiments were conducted at the National Agricultural Research Laboratories (NARL), Kawanda, from March 2013 to April 2017. Kawanda is located in Central Uganda at 32°36'E and 0°25'N, 1210 m above sea level (Tumuhimbise et al., 2016). During the experimental period, the mean annual rainfall was 1372 mm and temperatures ranged from 12.8 to 29.5°C. Kawanda is a hotspot for many pathogens and pests, including *Mycosphaerella fijiensis* Morelet, Foc race 1, weevils and nematodes.

Plant germplasm

Three genetically diverse parents sourced from the farmers' fields and NBRP (Table 1) were used to develop the two dessert banana genotypes that were evaluated for this study. Selection of the parents was based on their better performance for bunch yield, flowering-ability and relative degrees of field resistance to Foc race 1.

Genotype development

Targeted controlled crosses between male and female parental lines (Table 1) were made between 6:00 and 8:30 a.m. by dusting pollen on the stigmas of Sukali ndiizi. Before pollination, female and male flowers that had just flowered were bagged to avoid contamination by stray pollen. Seeds from mature ripe pollinated bunches were extracted, as described by Vuylsteke et al., (1995). *In vitro* germination of the extracted seeds was carried out according to the protocol described by Vuylsteke et al. (1990). The resulting seedlings were planted in the early evaluation trial at NARL in April 2011. Based on visibly high bunch yield, fruit size, fruit pulp smoothness, taste, color and smell, and resistance to black Sigatoka and Foc race 1 of the genotypes at the early evaluation trial stage (results not presented), two Sukali ndiizi hybrids (NAMU1 and NAMU2) were selected for further evaluation in a replicated preliminary yield trial. NAMU1 resulted from the cross Sukali ndiizi x Cultivar Rose, whereas NAMU2 resulted from the cross Sukali ndiizi x TMB2 x 8075-7. It is the results of these two new apple banana hybrids (NAMU1 and NAMU2) (Figure 1) and the two local check cultivars (Sukali ndiizi and Yangambi-KM5) that are presented and discussed in this paper.

Experimental design

Experiments were planted in a randomized complete-block design with three replications. Because of low multiplication rate of

Table 1. Three banana parental lines that were hybridized and their special attributes

Parent	Ploidy	Use	Source	Special attribute
TMB2×8075-7	2×	Male parent	NBRP	Resistant to Foc race 1, long bunch
Cultivar Rose	2×	Male parent	NBRP	Resistant to Foc race 1, firm fruit pulp and sweet
Apple banana (Sukali ndiizi)	3×	Female parent	Farmers' fields	Apple flavored, sweet, firm pulp, susceptible to Foc race1

NBRP: National Banana Research Program.

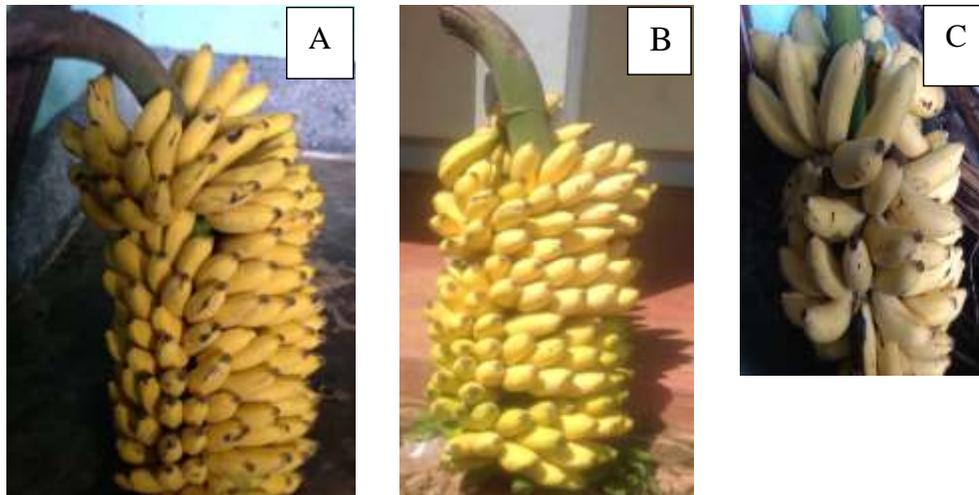


Figure 1. New apple banana genotypes: A= NAMU1, B=NAMU2, and their female parent: C=Sukali ndiizi.

bananas by suckers, NAMU1, NAMU2, Sukali ndiizi and Yangambi-KM5 were multiplied *in vitro* to generate sufficient planting materials for replicated preliminary yield trial. The tissue culture-derived plantlets of each genotype were planted in lines of 10 plants genotype⁻¹ replication⁻¹. Spacing between plants intra- and inter-lines was 3 × 3 m². At planting, 10 kg of Kraal manure was applied in 0.5 m deep and 0.6 m wide planting holes. The trial field had a history of severe Foc race 1 infestation, thus, considered a hot spot. Nevertheless, to avoid field escape of some plants to the pathogen because of uneven pathogen distribution, Foc race 1 inoculum at a concentration of 5 × 10⁶ spores/ml was distributed around the banana stools. Also, Sukali ndiizi, a banana cultivar susceptible to both Foc race 1 and black Sigatoka, was planted around the trial to act as a guard row, as well as a spreader for Foc race 1 and black Sigatoka.

Data collection

Data collection was done during the plant growth and at harvest on: plant height (cm), number of functional leaves at flowering, youngest leaf spotted at flowering as a measure of the genotypes' response to black Sigatoka, pseudostem splitting and corm discoloration. Harvesting of bunches was done when at least one fruit finger of the first hand on a bunch began to ripen and data were recorded on bunch weight (kg plant⁻¹), number of hands, fruit finger length (cm), and fruit finger circumference (cm). Bunch weight was obtained by weighing the harvested bunch using a weighing scale, whereas the number of hands on a bunch was

obtained by counting the hands on a bunch. Finger length was obtained by measuring the length of one middle finger from each hand on a bunch and the average length per bunch was calculated. Finger circumference was obtained by measuring the length around the middle finger of each hand on a bunch and the average circumference per bunch was calculated. Plant height was measured from the ground level to the point where the last leaf emerged from the pseudostem. Number of functional leaves was determined by direct counting of green leaves on a plant. Youngest leaf spotted at flowering was determined by recording the leaf number with the first black Sigatoka symptoms, counting from the youngest leaf to the oldest leaf. Resistance to Foc race 1, as determined by disease severity, was assessed based on internal corm symptoms and pseudostem splitting at harvest, as described by Smith et al. (2008) but with some modifications. Disease severity assessment based on corm symptoms was done using a scale of 0-6, where 0= no discoloration of tissue of stellar region of corm or surrounding tissue, 1 = no discoloration of stellar region of corm; discoloration at junction of root and corm, 2 = trace to 5% of stellar region discolored, 3 = 6-20% of stellar region discolored, 4 = 21-50% of stellar region discolored, 5 = more than 50% of stellar region discolored and 6 = discoloration of the entire corm stele. Disease severity assessment based on pseudostem splitting was done using a scale of 1-3, where 1 = no cracking of the pseudostem, 2 = slight cracking of the pseudostem and 3 = advanced cracking of the pseudostem.

For sensory traits evaluation, mature harvested bunches were stored in one of the laboratory rooms at NARL to ripen. The room temperature ranged from 23 to 29°C and relative humidity from 71

Table 2. Mean severity scores for pseudostem splitting and corm discoloration due to Foc race 1.

Genotype	Plant traits assessed [§]	
	Pseudostem splitting (1-3)	Corm discoloration (0-6)
Sukali ndiizi	2.06±0.1 ^a	4.76±0.2 ^a
Yangambi-Km 5	1.00±0.0 ^c	0.00±0.0 ^c
NAMU2	1.50±0.2 ^b	1.50±0.5 ^b
NAMU1	1.00±0.0 ^c	0.00±0.0 ^c
F prob.	≤0.0001	≤0.0001

[§]Means with different letters in the same column are significantly different by Fisher's protected least significant test at $\alpha = 5\%$ while those with the same letters are not significantly different.

Table 3. Mean performance of the genotypes for plant height, number of functional leaves and response to black Sigatoka.

Genotype	Plants traits assessed [§]			
	Plant height (cm)	Plant girth (cm)	NFLF	YLSF
Sukali ndiizi	263.57±3.8 ^c	42.70±0.6 ^c	9.56±0.2 ^b	7.95±0.2 ^b
Yangambi-Km 5	268.01±3.6 ^c	43.60±0.6 ^c	12.1±0.2 ^d	9.10±0.4 ^a
NAMU2	294.85±9.2 ^b	52.15±2.0 ^b	10.53±0.3 ^a	9.06±0.4 ^a
NAMU1	328.95±3.8 ^a	48.79±0.5 ^a	8.81±0.1 ^c	7.73±0.2 ^b
F prob.	<0.0001	<0.0001	<0.0001	0.013

NFLF: Number of functional leaves at flowering; YLSF: youngest leaf spotted at flowering. [§]Means with different letters in the same column are significantly different by Fisher's protected least significant test at $\alpha = 5\%$ while those with the same letters are not significantly different

to 81%. Fully ripened bunches were taken for sensory evaluation by a trained group of 20 apple banana farmers/consumers. Genotype assessments (NAMU 1, NAMU 2 and Sukali ndiizi) based on taste, color, smell and general acceptability were done on as a scale of 1 to 5, where 1 = dislike very much, 2 = dislike, 3 = like fairly, 4 = like, and 5 = like very much.

Statistical analysis

Data analysis was performed using SAS version 8.2 for windows (2001). To compare the trait means, Fisher's protected least significant test at $\alpha = 5\%$ was performed.

RESULTS

Genotypic response to Foc race 1

There were highly significant differences in mean scores of the experimental genotypes for corm discoloration and pseudostem splitting, as measures of Foc race 1 severity (Table 2). NAMU1 showed the highest resistance to Foc race 1, as it exhibited the lowest mean scores for pseudostem splitting and corm discoloration. Sukali ndiizi, the susceptible check cultivar, showed the highest mean scores for pseudostem splitting and corm discoloration.

Yangambi-KM5, the highly resistant check, showed the lowest mean scores for pseudostem splitting.

Genotypic performance relative to plant height, number of functional leaves and response to black Sigatoka

Highly significant differences were observed among the test genotypes for plant height, plant girth, number of functional leaves at flowering, and response to black Sigatoka (Table 3). NAMU1 showed the highest mean performances for plant height, followed by NAMU2; whereas Sukali ndiizi had the lowest plant height. NAMU2, on the other hand, had the highest mean performance for plant girth, followed by NAMU1. Sukali ndiizi had the lowest mean plant girth. Yangambi-KM5 and NAMU2 had the highest mean performance for the youngest leaf spotted.

Genotypic performance for bunch yield and yield-related traits

Highly significant differences were observed among the

Table 4. Mean performance of the genotypes for bunch yield, number hands, fruit finger circumference and fruit finger length.

Genotype	Plants traits assessed [§]			
	Bunch weight (kg plant ⁻¹)	Number of hands	Fruit finger circumference (cm)	Fruit finger length (cm)
Sukali ndiizi	6.22±0.3 ^a	6.12±0.1 ^a	10.06±0.1 ^a	11.17±0.2 ^a
Yangambi-KM5	6.55±0.3 ^a	6.41±0.1 ^a	10.21±0.1 ^a	11.37±0.2 ^a
NAMU1	9.85±0.3 ^b	7.45±0.2 ^b	13.60±0.2 ^b	14.30±0.1 ^b
NAMU2	10.38±0.7 ^b	8.57±0.4 ^c	12.13±0.5 ^c	12.88±0.3 ^c
F prob.	<0.0001	<0.0001	<0.0001	<0.0001

[§]Means with different letters in the same column are significantly different by Fisher's protected least significant test at $\alpha = 5\%$ while those with the same letters are not significantly different.

Table 5. Mean performance of the new hybrids and check cultivar for pulp smoothness, taste, color, smell and acceptability as assessed by farmer/consumers.

Genotype	Sensory attributes [§]				
	Smoothness	Taste	Colour	Smell	Acceptability
Sukali ndiizi	4.29±0.2 ^a	4.29±0.2 ^a	4.00±0.2 ^a	4.39±0.2 ^a	4.13±0.2 ^a
NAMU1	4.69±0.2 ^a	4.69±0.2 ^a	4.56±0.2 ^{ab}	4.63±0.2 ^a	4.38±0.2 ^a
NAMU2	5.00±0.3 ^a	4.69±0.3 ^a	4.94±0.2 ^b	4.50±0.3 ^a	4.60±0.2 ^a
F prob.	NS	NS	0.01	NS	NS

NS: Non-significant at 5%. [§]Means with different letters in the same column are significantly different by Fisher's protected least significant test at $\alpha = 5\%$ while those with the same letters are not significantly different.

test genotypes for the yield and yield-related traits (Table 4). The mean performances for bunch weight, number of hands, finger circumference and length were higher for the new apple banana genotypes than those of the check cultivars. For instance, the bunch weight was the highest for NAMU2, followed by NAMU1 and least for Sukali ndiizi. The number of hands was the highest for NAMU2, followed by NAMU1 and least for Sukali ndiizi. Fruit finger circumference, however, was the highest for NAMU1, followed by NAMU 2, and least for Sukali ndiizi. Fruit finger length was the highest for NAMU1, followed by NAMU 2 and the least for Sukali ndiizi.

Genotypes performance for fruit sensory traits

The new apple banana genotypes and check cultivar were only different for pulp color with NAMU1 having the highest score for consumer acceptability (Table 5). Genotypes was not different significantly for the pulp smoothness, taste, smell and general acceptability

DISCUSSION

The development of high-yielding and disease-resistant

bananas is essential for increased food security, improved human nutrition, and incomes for the farmers. Bananas are being improved for resistance to prevalent stresses, yield and quality by selecting for useful traits, and accumulating desirable genes from genetic resources.

In this research, the hybridisation of Sukali ndiizi with cultivar Rose and TMB2×8075-7 resulted in high-yielding, consumer-acceptable apple banana hybrids with resistance to Foc race 1. Hybridization introduced these agriculturally valuable traits into the progeny of Sukali ndiizi. Cultivar Rose and TMB2×8075-7 used in this breeding program are being used by other dessert banana breeding programs for *Fusarium* wilt resistance breeding.

Resistance of NAMU1 and NAMU2 to Foc race 1 gives assurance to farmers of sustained apple banana production and productivity. In addition, NAMU1 and NAMU2 showed partial and full resistances to black Sigatoka, respectively and good sensory attributes. Black Sigatoka, a leaf spot disease causes reduction in functional leaf area results in a decline in the quality and quantity of the fruit since the fruits of infected plants ripen prematurely before proper filling. The higher performance of NAMU1 and NAMU2 for the number of hands and fruits per bunch, as well as the fruit circumference and

length are of great economic importance to the producers and the market, as the bunch is the commercial unit of apple bananas. Moreover, fruit length and circumference are important criteria for selection of a commercial banana. In addition, of the four banana genotypes evaluated, NAMU1 and NAMU2 were characterized by the highest pseudostem girth, which is associated with plant vigor and cracking resistance of the pseudostem; thus, reflecting the support capacity for the plant and bunch. Genotypes with strong pseudostems are less susceptible to lodging by wind.

The ripe fruits' pulp smoothness, taste and smell of NAMU1 and NAMU2 were acceptable to the consumers and not significantly different from those of Sukali ndiizi. This implied that the new apple banana genotypes were as good as the local commercial Sukali ndiizi. Sensory attributes of crop plants are pertinent to their acceptability and adoption (Barrett et al., 2010). Therefore, since NAMU1 and NAMU2 are more yielding and resistant to Foc race 1 and black Sigatoka compared to Sukali ndiizi, they can be its perfect substitutes.

Conclusion

The results of this study show prospects for the use of hybrids that can substitute for Sukali ndiizi while increasing productivity not only in Uganda but also in other Sukali ndiizi-growing areas in the Great Lakes region of East Africa with similar production constraints, especially Foc race 1. As NAMU1 and NAMU2 combine resistance to Foc race 1 and black Sigatoka with improved yield and consumer-desired fruit characteristics, they can replace Sukali ndiizi that is adversely affected by Foc race 1. It is recommended that NAMU1 and NAMU2 can be evaluated in multi-location field trials to confirm their stability for yield and Foc race 1.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of heat stress on common bean under natural growing conditions in three locations in different climate zones in the state of São Paulo, Brazil

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Received 19 February, 2018; Accepted 12 April, 2018

Common bean (*Phaseolus vulgaris* L.) originated in medium to high altitude regions and is sensitive to high temperatures. Climate changes from an increase in global temperatures are foreseen, and therefore better understanding of the mechanisms of heat tolerance is necessary. In this context, the aim of this study was to investigate the effects of heat stress on twelve common bean genotypes under natural growing conditions in three locations (Campinas, Votuporanga, and Ribeirão Preto, SP, Brazil) and in two growing seasons (fall-winter 2016 and summer 2016/2017). Data were analyzed by combined analysis of variance in a $2 \times 3 \times 12$ factorial arrangement, considering two crop seasons, three locations, and twelve genotypes as factors. This was followed by the Scott-Knott mean comparison test ($P < 0.05$), genetics, genetics \times environment (GGE)-biplot analysis for grain yield and Pearson correlation for the summer season. Significant differences were found for the crop season, location, and genotype for most of the traits evaluated. It was found that the high temperatures, reached in summer, negatively affected the performance of cultivars, resulting in a reduction of 40% in grain yield. Votuporanga, which reached the highest temperatures during the summer, was considered as the most unfavorable environment. The genotypes that proved to be more productive in the summer for the locations of Campinas were BRS Agreste and FT Nobre; for Votuporanga, the genotypes Pérola and IPR Tangará; and for Ribeirão Preto, the genotypes SEA 5 and BRS Estilo. The highlighted correlations observed by the Pearson test were the highest leaf temperature reducing grain yield and, the highest relative index of chlorophyll contributed to higher productivity.

Key words: High temperature, *Phaseolus vulgaris*, selection, genotype \times environment interaction, plant breeding.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the main crops produced in Brazil and in the world. Its importance

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goes beyond economic factors considering its use as a basic food for the Brazilian population. According to CGIAR (2018), common bean is a highly nutritious food, containing protein, fiber, complex carbohydrates, vitamins and micro-nutrients. As such, beans strongly reinforce food and nutrition security among poor consumers, while also reducing the risk of cardio-vascular disease and diabetes. It is the most important grain legume for direct human consumption with 23 million hectares grown worldwide, and approximately 12 million metric tons produced annually.

Bean crop is grown in a wide range of latitudes with mean air temperature from 14 to 35°C, and due to its origin in medium to high altitude regions, it is sensitive to heat, whereas day and night temperatures above 30 or 20°C, respectively, result in significant yield reduction (Beebe et al., 2011). According to Araújo et al. (2015), common bean of Andean gene origin typically adapts better to cooler climate and high altitude (1400-2800 m) regions, whereas genotypes of Mesoamerican origin adapt to higher temperatures in low to medium altitude (400-2000 m) regions.

According to IPCC (2014), the surface temperature is projected to raise over the 21st century under all assessed emission scenarios. It is very likely that heat waves will occur more often and last longer, and that extreme precipitation events will become more intense and frequent in many regions. This increase in global temperature is most due to the continued emission of greenhouse gases and it will cause further warming and long-lasting changes in all components of the climate system, increasing the likelihood of severe, pervasive and irreversible impacts for people and ecosystems.

Thus, if there are no efforts to reduce carbon intensity in the atmosphere most crop areas of the world will be susceptible to high mean air temperatures, which may compromise agricultural production and food security, increasing the risk of drought, limiting and reducing rates of photosynthesis, interception of light, accelerating the phenological development and influencing the biomass, fruit, and grain production (Teixeira et al., 2013).

Souza et al. (2011), mentioned that an increase in temperature above the critical value for a sufficient period of time can cause irreversible damage, recognizing that the base temperature or tolerance limit of the plant may vary according to the species and the genotypes of the same species, and among the phenological phases of the same genotype. According to Talukder et al. (2014), thermotolerance in the field occurs under natural circumstances and, although high temperature is a frequently occurring phenomenon, little is known about the critical genes that control heat tolerance in plants. To maintain growth and yield, plants must adapt to stress conditions and activate specific tolerance mechanisms.

According to McClean et al. (2011), due to the short time available for changing the genetic composition of germplasm in the face of predictions of climate change,

efforts should be concentrated on the best understanding of the physiological mechanisms of tolerance to high temperatures and to water deficit, as well as on identification of genetic factors that control physiological responses to pyramid these factors in new cultivars leading to maximization of yield under drought alone and drought combined with heat stress.

In this context, the aim of this study was to investigate the effects of heat stress in twelve genotypes of common bean under natural growing conditions in three locations with different climate zones and in two crop season evaluating agronomical and morpho-physiological traits to test our hypothesis that the high temperatures reached in the summer season negatively affects the bean production and it is also possible to identify tolerant genotypes in these conditions.

MATERIALS AND METHODS

Field experiments were set up in the Grains and Fibers Center of the Instituto Agronômico - IAC (Santa Elisa Farm, Campinas, SP, Brazil), in the Polo Regional do Noroeste Paulista (Rubber Tree and Agroforest Systems Center, Votuporanga, SP, Brazil), and in the Polo Regional do Centro Leste (Sugarcane Center, Ribeirão Preto, SP, Brazil); all institutional bodies were connected with the Agência Paulista de Tecnologia do Agronegócio (APTA). The municipalities were chosen through their belonging to different climate zones, with medium to high temperatures, being presented in the climatic history of the last ten years (Table 1). Sowing was carried out in two crop seasons, fall-winter 2016 and summer 2016-2017, in order to synchronize the flowering period with the months of highest and lowest mean temperature.

Twelve common bean genotypes were used with different color of tegument and with different growth habits as I, II and III (upright determinate, indeterminate and prostrate indeterminate) (Table 2), being chosen considering their known performance for water deficit tolerance in regions where high temperatures occur, such as in the North of the State of São Paulo and in the Center-West of Brazil. A randomized block experimental design was adopted with four replications. Each experimental plot consisted of four four-meter rows, at a spacing of 0.5 m between rows and 0.1 m between plants.

The climate data regarding mean, maximum, and minimum temperatures during the growing period were acquired by the Centro integrado de informações agrometeorológicas - CIIAGRO ONLINE (<http://www.ciiagro.sp.gov.br/ciiagroonline/>).

In the period of full flowering (R6), four plants from the two center rows were sampled at random for the following evaluations.

Physiological evaluations

(1) Stomatal conductance (SC): A porometer (Type AP4 – Delta T Devices) was used, in a state of dynamic equilibrium. Readings were made between 9:00 and 11:00 in the morning on the abaxial surface of completely expanded leaves from the middle part of plants exposed to solar radiation.

(2) Leaf temperature (LT) was measured by an infrared thermometer (Telatemp model AG- 42D, Telatemp, Fullerton, CA, USA). The measurement was performed at 0.50 m from the leaf surface at an angle of 45° from the middle part of the plants; the readings were made at 9:00 in the morning.

(3) Relative chlorophyll index (RCI) was determined in the leaves

Table 1. Location, Koeppen climatic classification, climatic historical from 01/2008 to 12/2017 according to the Integrated Agrometeorological Information Center – CIIAGRO of the locations of Campinas, Votuporanga and Ribeirão Preto.

CAMPINAS							
Lat 22° 31m; Long 47° 2m; Alt 680 m							
Koeppen Climate Classification							
Cwa							
Month	TMAXA	TMINA	TMAXM	TMINM	TMED	ETP	PRECIP
January	33.59	15.97	29.15	19.10	24.15	137.6	263.98
February	33.83	17.43	30.61	19.67	25.14	127.9	152.6
March	32.92	15.93	29.09	19.00	24.07	115	182.32
April	31.66	12.78	27.94	17.30	22.62	83.5	83.53
May	29.34	9.98	25.12	14.18	19.64	61.5	63.54
June	28.36	7.95	24.00	12.58	18.29	49.4	70.68
July	29.32	7.53	24.85	12.45	18.63	55	30.46
August	32.19	9.27	26.74	13.44	20.1	69.6	28.7
September	34.31	11.08	28.47	15.56	22.02	90.1	60.39
October	35.35	13.12	29.22	17.5	23.37	117.4	89.3
November	33.63	14.94	29.09	18.2	23.65	121.9	163.54
December	33.72	16.27	29.73	19.25	24.5	143	197.08

VOTUPORANGA							
Lat 20° 15 m; Long 49° 34 m; Alt 525 m							
Koeppen Climate Classification							
Aw							
Month	TMAXA	TMINA	TMAXM	TMINM	TMED	ETP	PRECIP
January	35.6	18.44	31.49	20.87	26.19	161.7	260.03
February	35.66	19.1	32.18	20.87	26.53	141.2	180.21
March	38.98	17.4	31.79	20.47	26.14	140.6	181.46
April	40.73	8.74	30.75	18.03	24.37	103.1	58.38
May	32.27	9.03	28.53	15.19	21.88	74.6	48.59
June	32.11	7.65	27.72	14	20.88	60.7	33.32
July	32.63	7.9	28.73	13.87	21.31	66	20.4
August	35.6	8.24	30.88	14.92	22.89	85.5	18.78
September	37.59	12.23	32.48	17.31	24.91	114.6	70.78
October	38.46	14.22	32.86	19.24	26.05	149.3	89.94
November	36.26	16.49	32.13	19.96	26.06	151.9	136.69
December	35.95	18.12	32.03	20.92	26.48	161.6	183.05

RIBEIRÃO PRETO							
Lat 21° 6 m; Long 47° 28 m; Alt 531 m							
Koeppen Climate Classification							
Aw							
Month	TMAXA	TMINA	TMAXM	TMINM	TMED	ETP	PRECIP
January	33.76	17.32	29.91	19.48	24.71	140.9	245.36
February	34.07	17.51	30.87	19.51	25.21	128.3	160.03
March	33.19	16.41	29.72	18.93	24.34	118.7	167.48
April	32.14	12.07	28.99	17.2	23.11	87.5	59.24
May	30.15	8.84	26.61	14.24	20.43	66.4	53.21
June	29.53	7.26	25.91	12.88	19.4	55.3	35.32
July	30.37	7.3	26.76	12.74	19.76	60.9	16.54
August	32.95	8.05	28.88	13.92	21.4	76.4	21.2
September	35.58	10.9	30.76	16.3	23.52	100.2	61.57

Table 1. Contd.

October	36.71	13.16	31.37	18.29	24.83	132.9	85.76
November	34.73	15.09	30.43	18.71	24.56	132.7	166.17
December	34.18	15.73	30.33	19.45	24.89	143.7	210.04

ABMAXT- Absolute maximum temperature, ABMINT - Absolute minimum temperature, AVMAXT- Average maximum temperature, AVMINT- Average minimum temperature, AVT - Average temperature, PET - Potential evapotranspiration.

Table 2. Genotypes to be used in the study of heat tolerance.

Genotype	Grain type	Growth habit	Origin
1-SEA 5	Mulatto	Type I	CIAT
2-IAC Imperador	Carioca	Type I	IAC
3-SER 16	Red	Type I	CIAT
4- Pérola	Carioca	Type III	EMBRAPA
5-IAC Milênio	Carioca	Type III	IAC
6-FT Nobre	Black	Type II	FT-SEMENTES
7-BRS Estilo	Carioca	Type II	EMBRAPA
8-IAPAR 81	Carioca	Type II	IAPAR
9- IAC Diplomata	Black	Type II	IAC
10- IPR Tangará	Carioca	Type III	IAPAR
11- BRS Agreste	Mulatto	Type II	EMBRAPA
12-IAC Sintonia	Carioca	Type II	IAC

from the middle part of plant using the non-destructive method SPAD-502Plus (Konica Minolta) in the flowering stage (R6).

Morphological traits

- (1) Plant height (PH) in centimeters;
- (2) Number of nodes per plant (NNP);
- (3) Shoot dry matter (SDM) in grams;
- (4) Leaf area (LA) in cm², checked with the leaf area meter LI-COR (LI-3100C).

Agronomical traits

At physiological maturity, the two center rows of each plot were harvested to evaluate total grain yield (GY) and 100 seed weight (100SW) and three plants were sampled at random for the following evaluations: Number of pods per plant (NPP); Number of seeds per plant (NSP); Number of viable seeds per pod (NVSP); Number of aborted seeds per pod (NASP).

The experimental areas were irrigated in the absence of rainfall with the use of sprinklers. Soil moisture was kept at -40 kPa according to technical recommendation of the Watermark® measuring device. Crop treatments were made according to the needs of the crop.

The data were subjected to combined analysis of variance in a 2 × 3 × 12 factorial arrangement considering two crop seasons, three locations, and twelve genotypes as factors. This was followed by the Scott-Knott means comparison test at 5% probability, and GGE biplot analysis was performed to decompose the effects of the interactions among the factors for grain yield (GY). To verify the correlations between the variables, Pearson correlation analysis ($P > 0.05$) was performed considering only the data referring to the mean values of each variable in each location in the summer

crop season.

RESULTS AND DISCUSSION

According to Porch (2006), common bean is adapted to mild climate regions and, daytime and nighttime temperatures higher than 30 and 20°C, respectively, result in reduction of grain yield. The mean temperatures observed during the growing period in Campinas, Votuporanga, and Ribeirão Preto were 20.35, 21.27, and 22.88°C in the fall-winter season and 24.40, 26.26, and 23.87°C in the summer season, respectively. The peak of the absolute maximum temperatures reached in the summer, considered stressful to the common bean crop, were 33.5, 37.2, and 34.5°C, and the mean maximum temperatures were 31.35, 33.38, and 30.96°C for Campinas, Votuporanga, and Ribeirão Preto, respectively. This had a negative effect on genotype performance for grain yield. The Votuporanga environment in the summer crop with higher temperatures was the most unfavorable environment for grain yield (Figure 1). Analyses of variances (Table 3) for the crop season, location, and genotype factors showed significant effects for most of the traits studied. The significant effects of the blocks within the location were isolated from analysis for the traits leaf temperature (LT), stomatal conductance (SC), shoot dry matter (SDM), leaf area (LA), and grain yield (GY). Significant effects were also found for the crop

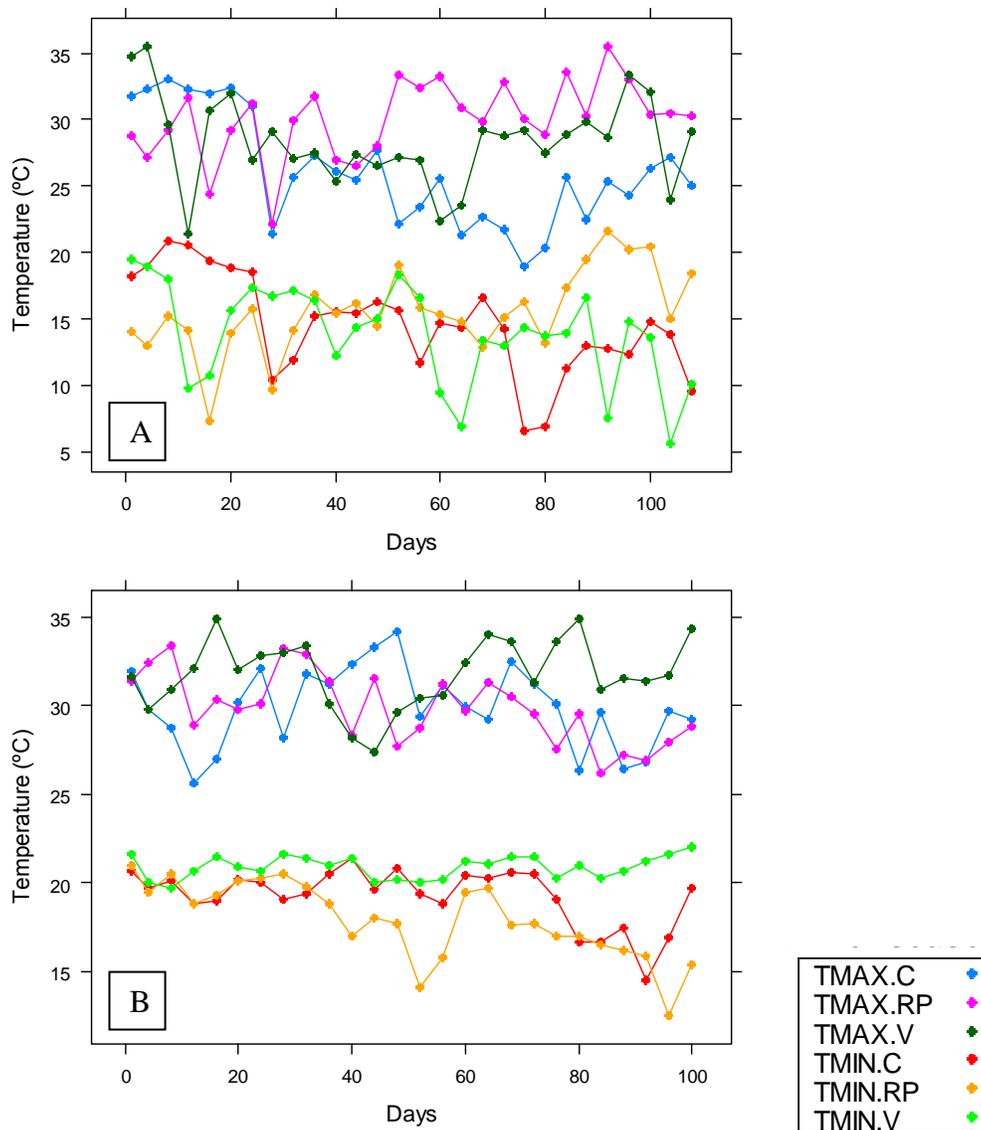


Figure 1. Absolute maximum and minimum temperatures of the environments of Campinas, Votuporanga, and Ribeirão Preto, SP, Brazil, registered over the crop cycle for each environment. A. Winter, B. Summer.

season \times location interactions in all the traits studied; effects of crop season \times genotype for plant height (PH), number of aborted seeds per plant (NASP), and 100 seed weight (100SW); effects of location \times genotype for PH, LA, NASP, 100SW, and GY; and effects of the triple interaction of crop season \times location \times genotype for relative chlorophyll index (RCI), PH, LA, number of viable seeds per plant (NVSP), NASP, and 100SW. The coefficients of variation, exhibiting low to medium magnitude, ranged from 5.98 to 30.13%, indicating good experimental precision.

The crop season factor significantly affected the performance of the traits, except for SDM and number of pods per plant (NPP) (Table 3). In the fall-winter, greater

development was observed of the LA characteristics and of the production and grain yield components number of seeds per plant (NSP), NVSP, NASP, 100SW, and GY. Thus, this crop season was more favorable to grain yield, with increases of 37.22, 5.01, 15.22, 36.45, 2.90, and 67.95% in these factors, respectively, in comparison to summer. In the summer, an increase in performance was observed in the characteristics LT, RCI, PH, NNP, SC, SDM, and NPP of 30.76, 7.69, 12.15, 10.53, 123.32, 9.27, and 5.21%, respectively, in comparison to performance in the winter crop season.

Uddin et al. (2007) studying the seasonal influence on yield and yield components characters of four lablab bean genotypes covering one main season (winter) and

Table 3. Summary of analyses of variance in relation to cultivation of twelve genotypes of common bean grown in the winter 2016 and summer 2016/2017 crop seasons in Campinas, Votuporanga, and Ribeirão Preto, SP, Brazil.

Source of variance	DF	LT	RCI	SC ^t	PH	NNP	SDM ^t	LA	NPP ^t	NSP ^t	NVSP	NASP ^t	100SW	GY
Mean square														
Crop season	1	2298.3**	468.7**	3092**	4386**	119.43**	0.02	64526687**	0.099	14.86*	24.44**	1.2524**	32.3**	33487544**
Location	2	640.2**	1543.2**	748.2**	16671**	90.52**	34.27**	21236758**	15.487**	69.93**	1.44	0.177**	912.2**	10593267**
Genotypes	11	2.8	143.9**	10.9	4711**	30.54**	0.33	1565274**	1.455**	2.95	3.85**	0.097**	132.2**	285626
Location:Block	9	11.2**	21.4	52.6**	142	9.48	0.7*	2337088**	0.407	1.14	0.66	0.0417	0.6	389657*
Crop season: Location	2	223.5**	367.3**	1514.1**	20465**	20.64*	55.38**	54264197**	10.597**	169.28**	39.69**	0.1411**	2007.1**	53888634**
Crop season:Genotypes	11	2.5	31.7	5.6	351*	5.93	0.19	911224	0.523	2.96	0.61	0.0714**	9.8**	220859
Location:Genotypes	22	1.7	20.7	8.3	303**	7.78	0.38	1237468**	0.611	3.56	0.71	0.0663**	10.1**	392903**
Crop season:Location:Genotypes	22	1.5	31*	9	529**	5.76	0.47	1165877*	0.432	2.13	0.87*	0.0444**	7.7**	157051
Residuals	207	3	17.7	15.1	156	5.78	0.33	640082	0.518	2.59	0.49	0.0223	2	162347
CV (%)	-	8.13	12.22	21.39	18.34	18.78	14.77	26.52	17.9	20.42	17.12	10.02	5.98	30.13

^tData transformed $\sqrt{x+1}$. DF: degree of freedom; LT: leaf temperature (°C); RCI: relative chlorophyll index (SPAD); CS: stomatal conductance (mmol m⁻² s⁻¹); PH: plant height (cm); NNP: number of nodes per plant; SDM: shoot dry matter (g); LA: leaf area (cm²); NPP: number of pods per plant; NSP: number of seeds per plant; NVSP: number of viable seeds per pod; NASP: number of aborted seeds per pod; 100SW: 100 seed weight (g); GY: grain yield (g).

two off-seasons (early summer and late summer) also verified that all the genotypes performed better for all the parameters during winter. Pod setting was reduced during late summer in all the genotypes as the number of seeds per pod. They also verified that most of the agronomic traits as number of inflorescence per plant, number of flower buds per inflorescence, number of pod set per inflorescence, number of pod per plant, and single pod weight were severely affected.

Román-Avilés and Beaver (2003) also observed the high temperature influence in the common bean production studying the inheritance of heat tolerance in Andean genotypes and, observing that the Indeterminate Jamaica Red and DOR 303 genotypes presented, respectively, the double and 4 times greater mean of seed yields in the winter season than the summer season. However, the performance of the other lines in the trials suggests that selection for seed yield in the winter

months would not guarantee the identification of high-yielding lines for the summer months. Selection for adaptation to high temperature environments requires the evaluation of bean lines during the summer months.

According to Kaushal et al. (2016), heat stress has harmful effects on plants, affecting growth, development, metabolism, and yield. Exposure to high temperatures causes a series of morpho-anatomical, physiological, and biochemical changes, reducing the life cycle, increasing senescence, and severely affecting yield. A mean temperature of 21.3°C in the winter and 24.84°C in the summer and a maximum absolute temperature of 32.31°C in the winter and 35.06°C in the summer were registered in this study. Thus, these temperatures negatively affected the performance of cultivars in the summer crop season, resulting especially in higher leaf temperature, with a mean increase of 5.65°C and,

consequently, less development of leaf area and of production components and mean reduction of 40% in grain yield.

According to Kumar et al. (2015), lower temperature of the plant canopy is frequently associated with higher grain yield, with a deeper root system, and with greater stomatal conductance in environments subjected to high temperature. Therefore, selection for temperature of the plant canopy, combined with greater initial vigor and delayed senescence to improve interception of light, as well as greater stability of the membrane, the presence of photoprotective pigments, and wax to improve the efficiency of the use of radiation, are desirable for making selection for heat tolerance.

The heat stress intensity index was verified according to Fisher and Maurer (1978), considering the fall-winter crop season as non-stressful and summer as stressful, for the combined

data and for the locations of Campinas, Votuporanga, and Ribeirão Preto, obtaining indexes of 0.40, 0.14, 0.82, and -0.47%, respectively. Thus, the Votuporanga location, which reached the highest temperatures during the summer crop season, had the highest stress intensity index, and it was considered drastic, reducing the mean yield from 2885 kg.ha⁻¹ observed in fall-winter to 513 kg.ha⁻¹ in summer. A lower heat stress intensity index was also found for Campinas. This may be explained by the low yield also achieved in the fall-winter crop season of 1124 kg.ha⁻¹, in which a mean minimum temperature of 13.5°C and an absolute minimum of 5.5°C were observed, while in the summer, a grain yield of 968 kg.ha⁻¹ was observed. However, a negative stress intensity index was registered in Ribeirão Preto since the mean yield achieved by the genotypes in the winter season was 1026 kg.ha⁻¹, less than the yield observed in the summer, of 1508 kg.ha⁻¹. The mean, mean maximum, and absolute maximum temperatures reached in the summer were 23.87, 30.96, and 34.5°C, respectively. In addition, in that season, the mean of the maximum temperatures was the lowest among the locations, which favored the highest grain yield in the summer among the locations.

Porch (2006) found a heat stress intensity index of 0.66 in cultivation of 14 genotypes in two locations with high and low temperature in field experiments, and an index of 0.98 in experiments evaluating the same genotypes in a greenhouse. The mean temperatures in the different environments ranged from 25.2 to 29.2°C. According to the author, due to the higher heat stress intensity observed in the experiments in the greenhouse, these experiments were less informative and, furthermore, it reinforced that moderate indexes are more adequate for differentiation of the genotypes.

For the location factor, significant effects were also observed for the characteristics, except for NVSP. In location 1, Campinas, the variables LT, and NASP exhibited their highest mean values, which were 24.08°C and 1.39 aborted seeds, respectively. In location 2, Votuporanga, the best mean performances were found for RCI, SC, LA, NVSP, and GY, with values of 37.4 SPAD units, 493.86 (mmol m⁻² s⁻¹), 3425.66 cm², 4.22 viable seeds per pod, and 1699.0 kg.ha⁻¹, respectively. In location 3, Ribeirão Preto, the variables that exhibited the best mean performances were PH, NNP, SDM, NPP, NSP, and 100SW, with values of 83.40 cm, 13.92 nodes.plant⁻¹, 21.16 g, 4.48 pods.plant⁻¹, 83.10 seeds.plant⁻¹, and 26.94 g (Table 4).

For the genotype factor, significant effects were detected for the variables RCI, PH, NNP, LA, NPP, NVSP, NASP, and 100SW, showing variability among the genotypes for the characteristics evaluated. There was variation from 37.9 to 30.46 SPAD units for the RCI variable, and the genotypes that exhibited the highest mean values were Pérola, IAC Milênio, IPR Tangará, and FT Nobre. PH exhibited mean variation from 95.14 to 49.47 cm, and the genotypes with the greatest and

smallest height were Pérola and IAC Imperador, respectively. The mean values exhibited for NNP were 14.33 to 9.98 and nine genotypes exhibited more than 13.49 nodes per plant. Mean production in regard to LA ranged from 3311.61 to 2454.83 cm². Eight genotypes stood out with production greater than 3000 cm², and the highest and lowest LA values found were for the genotypes FT Nobre and IAC Imperador, respectively.

Siddiqui et al. (2015) also observed significant differences in ten bean genotypes that were subjected to a control and two high temperature treatments (25, 31, and 37°C) evaluating morphophysiological traits. Data revealed that the growth attributes of all the genotypes were significantly affected by temperature in all the cultivars. Plant height, shoot dry and fresh matter, leaf area, and total chlorophyll synthesis exhibited gradual reductions from the control to the treatments with high temperature. The decrease observed in these parameters was attributing to loss of turgidity, altering cell division and lengthening, and reduction in total biosynthesis of chlorophyll due to inhibition of photosynthetics in the electron transport chain.

Mean production of NPP ranged from 18.58 to 11.18. Ten genotypes exhibited mean values higher than 14 pods per plant, and the highest and lowest mean values observed were for IAC Sintonia and SEA 5, respectively. The NVSP ranged from 4.67 to 3.48, eight genotypes exhibited values of more than four seeds per pod, and the genotypes with the highest and lowest number of pods were SEA 5 and IAC Sintonia, respectively. For NASP, there was a mean from 1.51 to 0.77 aborted seeds, with only the genotype SEA 5 standing out with the lowest index of seed abortion. In relation to 100 seed weight, mean values from 26.92 to 18.35 were found, highlighting the genotypes with highest and lowest weight, IAC Milênio and IAC Sintonia, respectively (Table 3). Porch et al. (2010) field evaluated two genotypes of heat tolerant common bean, TARS-HT1 (PI 98059-6-2-1) and TARS-HT2 (PI 98059-10-2-1), and the lines resulting from hybridization between the two genotypes under mild conditions and under high temperature in two climate zones and in two seasons of the year. These authors found that the genotype TARS-HT1, one of the parents, proved to be the genotype most tolerant to heat among the 24 tested in the trials, showing 0% reduction in number of pods and reduction of 22% in number of seeds under high nighttime temperature conditions, compared to the treatment without stress.

Rainey and Grif (2005) assessed the production components of 24 common bean genotypes after exposure to four treatments of daytime/nighttime temperature (24/21, 27/24, 30/27, and 33/30°C). The treatment with the highest temperature showed decreases in number of seeds, number of pods, seed weight, and seed/pod weight of 83, 63, 47, and 73% on average, respectively. The heat tolerant genotypes showed different responses to high temperatures, suggesting

Table 4. Mean performance (Scott-Knott 5%) in relation to cultivation of twelve genotypes of common bean grown in the fall-winter 2016 and in the summer 2016/2017 crop seasons in Campinas, Votuporanga, and Ribeirão Preto, SP, Brazil.

Crop season	LT	RCI	SC	PH	NNP	SDM	LA	NPP	NSP	NVSP	NASP	100SW	GY
1	18.38 ^b	33.15 ^b	229.07 ^b	64.30 ^b	12.15 ^b	14.34 ^b	3489.63 ^a	15.45 ^a	66.91 ^a	4.39 ^a	1.46 ^a	23.75 ^a	1678.23 ^a
2	24.03 ^a	35.70 ^a	511.54 ^a	72.10 ^a	13.44 ^a	15.67 ^a	2542.95 ^b	16.30 ^a	63.72 ^a	3.81 ^b	1.07 ^b	23.08 ^b	996.25 ^b
Location													
1	24.08 ^a	36.01 ^b	247.35 ^c	61.05 ^b	12.26 ^a	12.10 ^b	2502.53 ^c	13.81 ^b	55.82 ^b	3.97 ^a	1.39 ^a	22.07 ^b	1045.94 ^c
2	19.09 ^c	37.40 ^a	493.86 ^a	60.14 ^b	12.21 ^b	11.76 ^b	3425.67 ^a	13.69 ^b	57.02 ^b	4.21 ^a	1.27 ^a	21.23 ^c	1699.00 ^a
3	20.43 ^b	29.87 ^c	369.70 ^b	83.41 ^a	13.92 ^a	21.16 ^a	3120.67 ^b	20.13 ^a	83.10 ^a	4.12 ^a	1.14 ^b	26.94 ^a	1266.79 ^b
Genotype													
1	20.99 ^a	32.61 ^c	445.90 ^a	56.58 ^d	11.82 ^b	17.61 ^a	3189.71 ^a	11.18 ^b	52.29 ^a	4.67 ^a	0.77 ^b	25.97 ^b	1560.79 ^a
2	21.65 ^a	32.48 ^c	328.38 ^a	49.47 ^e	9.99 ^c	15.46 ^a	2454.83 ^b	17.71 ^a	63.79 ^a	3.61 ^b	1.51 ^a	21.53 ^e	1258.05 ^a
3	21.75 ^a	30.47 ^c	368.85 ^a	59.69 ^d	12.07 ^b	15.86 ^a	3025.18 ^a	17.83 ^a	66.75 ^a	3.59 ^b	1.23 ^a	22.74 ^d	1305.41 ^a
4	21.08 ^a	37.90 ^a	323.82 ^a	95.14 ^a	14.33 ^a	13.57 ^a	2740.25 ^b	13.79 ^b	60.21 ^a	4.29 ^a	1.40 ^a	24.50 ^c	1328.37 ^a
5	20.99 ^a	37.76 ^a	344.50 ^a	82.31 ^b	12.97 ^a	14.01 ^a	2879.44 ^b	14.92 ^a	55.63 ^a	3.77 ^b	1.19 ^a	26.92 ^a	1297.32 ^a
6	21.11 ^a	35.88 ^a	370.14 ^a	57.93 ^d	13.38 ^a	15.08 ^a	3311.61 ^a	16.33 ^a	70.79 ^a	4.53 ^a	1.21 ^a	18.35 ^f	1223.40 ^a
7	21.60 ^a	34.56 ^b	389.22 ^a	55.32 ^d	13.43 ^a	14.27 ^a	3268.14 ^a	15.58 ^a	71.21 ^a	4.38 ^a	1.15 ^a	23.14 ^d	1386.25 ^a
8	21.44 ^a	33.85 ^b	378.35 ^a	62.19 ^c	12.49 ^a	15.02 ^a	3106.93 ^a	17.25 ^a	69.46 ^a	4.02 ^a	1.33 ^a	23.22 ^d	1297.47 ^a
9	20.67 ^a	34.80 ^b	387.39 ^a	69.47 ^c	12.76 ^a	15.81 ^a	2780.82 ^b	15.29 ^a	66.71 ^a	4.32 ^a	1.36 ^a	21.68 ^e	1175.45 ^a
10	21.27 ^a	37.24 ^a	355.69 ^a	84.88 ^b	13.36 ^a	13.68 ^a	3097.78 ^a	16.25 ^a	71.83 ^a	4.41 ^a	1.22 ^a	25.33 ^c	1502.14 ^a
11	21.01 ^a	31.35 ^c	376.95 ^a	67.81 ^c	13.75 ^a	14.56 ^a	3104.56 ^a	15.75 ^a	68.04 ^a	4.16 ^a	1.33 ^a	22.39 ^d	1334.24 ^a
12	20.87 ^a	34.25 ^b	374.49 ^a	77.63 ^b	13.21 ^a	15.16 ^a	3236.22 ^a	18.58 ^a	67.08 ^a	3.48 ^b	1.46 ^a	25.18 ^c	1378.02 ^a

DF: Degree of freedom; LT: leaf temperature (°C); RCI: relative chlorophyll index (SPAD); CS: stomatal conductance (mmol m⁻² s⁻¹); PH: plant height (cm); NNP: number of nodes per plant; SDM: shoot dry matter (g); LA: leaf area (cm²); NPP: number of pods per plant; NSP: number of seeds per plant; NVSP: number of viable seeds per pod; NASP: number of aborted seeds per pod; 100SW: 100 seed weight (g); GY: grain yield (g).

differential genetic control of the heat tolerance mechanisms, and the authors indicated the treatment at 30/27°C as the optimum treatment for selection of materials for heat tolerance.

GGE-biplot analysis was carried out for the grain yield and, the Figure 2 shows that the six environments are divided in the biplot in four sectors, showing high correlation among the environments of Ribeirão Preto in fall-winter, Ribeirão Preto in the summer, and Campinas in

the fall-winter (PGIRP, PGVRP, and PGIC), with grain yield of 1025.50, 1508.08, and 1123.93 kg.ha⁻¹, respectively, and the other sectors represent the environments of Campinas in the summer (PGVC) with mean yield of 969.93 kg.ha⁻¹, Votuporanga in the summer (PGVV) with mean yield of 512.74 kg.ha⁻¹, and Votuporanga in the fall-winter (PGIV) with mean yield of 2885.26 kg.ha⁻¹.

GGE-biplot analysis also shows that the most

efficient genotypes in the summer for the Campinas locations were 11 - BRS Agreste (1121.356 kg.ha⁻¹) and 6 - FT Nobre (961.3063 kg.ha⁻¹); for Votuporanga, the genotypes 4 - Pérola (921.2375 kg.ha⁻¹) and 10 - IPR Tangará (660.5313 kg.ha⁻¹); and for Ribeirão Preto, the genotypes 1 - SEA 5 (2065.75 kg.ha⁻¹) and 7 - BRS Estilo (1486.268 kg.ha⁻¹). It can be inferred that the genotypes BRS Pérola and IPR Tangará were those that exhibited the best heat tolerance

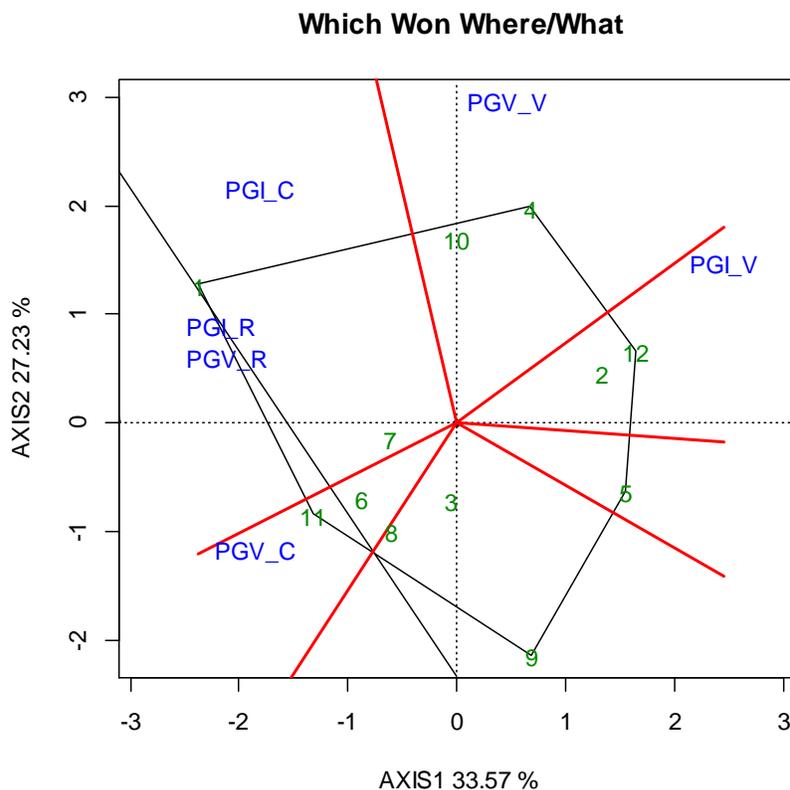


Figure 2. GGE-biplot in relation to grain yield ($\text{kg}\cdot\text{ha}^{-1}$) of twelve common bean genotypes grown in the winter 2016 and in the summer 2016/2017 crop seasons in Campinas, Votuporanga, and Ribeirão Preto, SP, Brazil.

since they exhibited the highest yields under high temperature conditions in the environment of Votuporanga in the summer (PGVV), which was the environment that exhibited the highest mean temperature (27°C) and the highest absolute maximum mean value (37°C) among all the environments.

The genotypes SEA 5 and BRS Estilo were the best in grain yield also for the environments Ribeirão Preto and Campinas in the fall-winter (PGIRP PGIC). These results corroborate Pereira et al. (2012), who, in their studies, identified the cultivar BRS Estilo with high grain yield in the dry and winter crop seasons in regions of the *Cerrado* (Brazilian tropical savanna) in the state of Mato Grosso. In the environment of Votuporanga in the fall-winter, which exhibited higher yield than the other environments ($2885.26 \text{ kg}\cdot\text{ha}^{-1}$), the genotypes IAC Sintonia ($3401.25 \text{ kg}\cdot\text{ha}^{-1}$) and IAC Imperador ($3157.5 \text{ kg}\cdot\text{ha}^{-1}$) stood out with the highest mean yields.

According to Didonet (2010), high temperature may be the environmental factor that has the greatest influence on flower abscission, low setting and final retention of pods, inadequate grain filling, reduction in the number of seeds per pod, and lower seed weight in common bean. This corroborates the results found in these experiments since the production components and grain yield were

affected by crop season, thus showing the differential behavior of the genotypes when exposed to different growing environments.

Around 1000 lines of common bean (including Andean and Mesoamerican groups, interspecific crosses, and advanced lines) were evaluated for heat tolerance under field conditions in Armero, Colombia, by the Plant Breeding Program of CIAT (International Center for Tropical Agriculture), where the temperatures during the crop season considered stressful were 35°C for maximum temperature and 23°C for minimum temperature and 22.8°C for nighttime temperature. In this study the germplasm tested for heat tolerance proved to be very sensitive; nevertheless, it was possible to identify 40 superior genotypes based on visual observation in regard to pod formation. The authors infer that better pod formation observed in these genotypes occurred due to the presence of viable pollen and, consequently, successful pollination, as well as due to the differences observed in grain filling (CGIAR, 2015).

Pearson correlation analyses were performed (Table 5) among all the variables studied considering the mean values obtained in the three locations in the summer crop season. Thus, Table 5 shows the presence of negative and highly significant correlations at 1% probability

Table 5. Pearson correlation relative to cultivation of twelve genotypes of common bean grown in the winter 2016 and in the summer 2016/2017 crop seasons in Campinas, Votuporanga, and Ribeirão Preto, SP, Brazil.

Correlation	RCI	PH	NNP	SC	LA	SDM	NPP	NSP	NVSP	NASP	100SW	GY
LT	-0.2045	-0.0817	0.0067	0.3298*	0.209	0.2834	0.021	0.0934	0.065	0.1551	-0.4868**	-0.5141**
RCI		0.4016*	0.311	0.3484*	0.538**	0.1323	-0.0641	0.3933*	0.6161**	-0.1255	0.3482*	0.6089**
PH			0.4754**	-0.0893	0.2804	0.1536	0.0228	0.0035	-0.029	0.1451	0.3004	0.1013
NNP				-0.1526	0.178	-0.1813	0.1039	-0.0058	-0.1263	-0.0557	-0.0874	-0.2183
SC					0.4774**	0.3375*	-0.1465	0.3158	0.5707**	0.0217	-0.0676	-0.0652
LA						0.6211**	0.1296	0.5658**	0.5958**	-0.0318	-0.1315	0.2293
SDM							0.1167	0.383*	0.3723*	-0.0742	-0.1259	0.0948
NPP								0.6749**	-0.2625	0.2797	-0.1493	-0.2028
NSP									0.5144**	-0.0507	-0.1736	0.0603
NVSP										-0.3865*	-0.0662	0.3307*
NASP											-0.1922	-0.1806
100SW												0.6502**

DF: Degree of freedom; LT: leaf temperature (°C); RCI: relative chlorophyll index (SPAD); CS: stomatal conductance (mmol m⁻² s⁻¹); PH: plant height (cm); NNP: number of nodes per plant; SDM: shoot dry matter (g); LA: leaf area (cm²); NPP: number of pods per plant; NSP: number of seeds per plant; NVSP: number of viable seeds per pod; NASP: number of aborted seeds per pod; 100SW: 100 seed weight (g); GY: grain yield (g).

between the LT variables and 100SW (-0.4868) and LT and GY (-0.5141). In other words, the higher temperature in the leaves of the plant canopy hurt pod filling, resulting in lower grain yield, as well as formation of lower weight grain. In addition, negative interaction was found between the number of viable seeds (NVSP) and the number of aborted seeds (NASP).

Twenty positive correlations were observed: LT with SC (0.3298), RCI with PH (0.4016), RCI with SC (0.3484), RCI with LA (0.538), RCI with NSP (0.3933), RCI with NVSP (0.6161), RCI with 100SW (0.3482), PH with NNP (0.4754), SC with LA (0.4774), SC with SDM (0.3375), SC with NVSP (0.5707), LA with SDM (0.6211), LA with NSP (0.5658), LA with NVSP (0.5958), SDM with NSP (0.383), SDM with NVSP (0.3723), NV with NSP (0.6749), NSP with NVSP (0.5144), NVSP with GY (0.3307), and 00SW with GY (0.6502).

The correlations observed between leaf temperature and stomatal conductance corroborate the plant responses described by Sicher and Bunce (2015) in which, CO₂ enrichment is able to attenuate the effects of moderate heat stress in plants that have the C₃ photosynthesis pathway, and mitigation of heat stress declines as temperatures increase. According these authors, high concentrations of CO₂ induce stomatal closing in many plant species, reducing the rates of leaf evapotranspiration, and the higher leaf water potential would benefit plants in the field during prolonged exposures to heat stress and high air temperatures create a demand for lower leaf temperatures, inducing stomatal opening and an increase in evapotranspiration rates. Thus, very high temperatures block the effects of CO₂ enrichment on stomatal opening and the growth of plants in high CO₂ concentration, that is, interfering at the beginning of senescence of various annual crops.

Furthermore, positive correlations were also found between stomatal conductance and more extensive formation of leaf area, shoot dry matter, and a higher number of viable seeds. According to Pimentel et al. (2013), in addition to biochemical limitation to photosynthesis, carbon supply is also physically limited on the leaf surface, through stomatal conductance (gs) and through mesophyll conductance (gm). Under high temperatures, diffusion limitations may occur in which gs, the main regulatory control that limits CO₂ diffusion in the leaf, is affected by the differences in atmospheric vapor pressure deficit (VPD), and gm is affected both by metabolic activity and by leaf anatomy.

Porch and Hal (2013) also report the occurrence of a positive correlation between grain yield and photosynthetic rate, and also between grain yield and stomatal conductance in spring wheat growing in hot. This indicates that more fully open stomates of heat resistant cultivars may lead to improved photosynthesis, facilitating diffusion of CO₂ in the leaves and increasing transpirational cooling, that is, creating temperatures lower than the damage threshold. In addition, yield differences of a cultivar in a hot and irrigated environment were positively correlated with the number of kernels per spike.

The relative chlorophyll index checked in the middle third of the plants showed positive correlations with the plant shoot traits of PH and LA, the physiological trait of SC, and with the production components of NSP, NSV, 100SW, and GY. According to Signorelli et al. (2015), various studies have reported correlation between changes in the parameters of chlorophyll fluorescence in response to environmental stresses such as high temperature, reducing the quantity of photosynthetic pigments, reduces photosynthetic and respiratory activity

of the plant. Thus, the positive correlations observed between the relative chlorophyll index contributed significantly to an increase in grain yield and to better performance of genotypes in the summer crop season.

Greater development of the morphological parameters LA and SDM also contributed to greater development of seeds per plant, just as to a higher number of viable seeds per pod. This behavior was expected since the increase in photosynthetic area resulted positively in higher production of photosynthates and, consequently, better development of the production components.

Pearson correlation analyses allowed assessment of the relations among the variables that in the summer crop season contributed to the performance of the cultivars exposed to high temperatures.

Conclusions

The crop season factor significantly influenced the performance of genotypes and the high temperatures observed in the summer crop season drastically reduced the grain yield of the cultivars, and the mean heat stress intensity index was 0.4;

The environment Votuporanga in the summer season was the most unfavorable environment, reaching the highest absolute average temperatures, which resulted in a grain yield reduction of 82.2%.

Due to the high interaction of genotype vs. location and season vs. location for grain yield, it was observed that these genotypes do not have wide adaptability for high temperature, being necessary to carry out the evaluations and selections in unfavorable environments, as Votuporanga.

The genotypes that proved to be more productive in the summer for the locations of Campinas were BRS Agreste and FT Nobre; for Votuporanga, the genotypes Pérola and IPR Tangará; and for Ribeirão Preto, the genotypes SEA 5 and BRS Estilo.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors thank for financial support: FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo. CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico

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Full Length Research Paper

Genetic variation of tef [*Eragrostis tef* (Zucc.) Trotter] genotypes for reaction to tef shoot fly [*Atherigona hyalinipennis* Van Emden], at Maysiye, Northern Ethiopia

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Received 24 November, 2017; Accepted 29 January, 2018

One of the major biotic stresses that limits tef yield is insect pests attack. Of these tef, shoot fly is sporadically important insect pest in various tef growing areas of Ethiopia. Evaluation of diversity of tef germplasm in Ethiopia for the reaction tef shoot fly might give a chance to get host plant resistance. The main objective of this study was to assess the genetic variation among tef genotypes for their reaction to tef shoot fly. Field experiment was conducted in 2015 cropping season at Maysiye, Northern Ethiopia. The genotypes were planted in triple lattice design with three replications category of reaction of the tested tef genotypes for their reaction to shoot fly attack was adopted from the rating scale of the IRRI for rice. The study revealed that acc. 17 WJ was found to be the only genotype evaluated as resistant to tef shoot fly *Atherigona hyalinipennis* species. While the standard checks Quncho and Kora were grouped under the susceptible category, generally 10 tef genotypes were moderately resistance, 19 genotypes were moderately susceptible, 16 genotypes were susceptible, and three genotypes were highly susceptible to tef shoot fly attack. Tef damage (dead heart and white headed) due to tef shoot fly ranged from 4.20 to 36.96%, respectively. The yield loss also varied from 3.58 to 97.83 kg ha⁻¹. Infestation was high from the beginning of August to the end of September. Besides using the varieties grouped under resistance and moderately resistance category and chemical application at early stage infestation would be recommended to avoid yield loss. Further study over seasons and environments would be important to have a valid conclusion

Key words: Shoot fly resistance, time of infestation, yield loss, tef genotypes.

INTRODUCTION

Tef (*Eragrostis tef* (Zucc.) Trotter, is one of the major staple cereals of Ethiopia. Ethiopia is the center of both origin and diversity for tef (Vavilov, 1951). It occupies

more than three million hectares of land and considered to be a healthy food as its gain is gluten free (Spaenij-Dekking et al., 2005) and the crop is very important in the

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overall national food security of the country (Kebebew et al., 2013).

In spite of the fact that tef has numerous merits and considerable economic significance in Ethiopia, the national average grain yield of tef is relatively low, about (1575 kg ha⁻¹) (CSA, 2015). However, Tareke et al. (2013) reported that the tef yields of 4000 and 2500 kg ha⁻¹ on research fields and on farmers' fields, respectively.

Tef's major yield limiting factors are the low yield potential of tef landrace, lack of cultivars tolerant to lodging, drought and pests (Assefa et al., 2011). One of the major biotic stresses that limits tef yield is insect pests attack. Among the 40 insect pest species recorded on tef, tef shoot fly (*Atherigona hyalinipennis* Van Emden) is sporadically important insect pest in various tef growing areas (Sileshi, 1997). Tef productions in areas with erratic rainfall distribution like northern part of Ethiopia is severely affected by tef shoot fly infestation. This might be due to the conducive climate for the reproduction of the tef shoot fly, weakening of tef plant due to drought effect and perhaps changes in agronomic practice (fertilizer application, use of improved varieties).

Tef is infested by six shoot fly species that belonged to three families and three genera. Of these the three species belonged to the family Muscidae and the genus *Atherigona*. *A. hyalinipennis*, *Atherigona Lineata* (Adams) ssp. and *Atherigona longifolia* Van Emden are the family which belongs to Muscidae. The second family is the Anthomyiidae of the *Delia flavibasis* species. Whereas, the third family Chloropidae flies, *Oscinella nartshukiana* Beschovski and *Oscinella* sp. n. *dimidiofrit* were another group of shoot flies that caused dead heart in tef. The species *A. hyalinipennis* was previously reported as pest of tef by Ebba (1969) and Sileshi (1997).

Taxonomic history, description, identification key and host plants of these *Atherigona* spp. is found in Deeming (1971). The other tef shoot fly species is *D. flavibasis* (Stein in Becker), which belongs to the family Anthomyiidae, is also a new record on tef under field condition.

In areas depending up on season and location shoot fly damage on tef ranged from 6.96 to 37.60% in Tigray region and 2.98 to 22.87% in Awi zone (DZARC, 2004). Moreover, in north Wollo and Wag-hmra zone damaged tef panicles in 25×25 cm quadrant were in the range of 2 to 4% (Bayeh, 2004). However, tef shoot fly damage may not always lead to yield loss. Thus, where rain fall is plenty, tef compensates for shoot fly damage and grain yield from insecticide unsprayed tef was greater than grain yield from insecticide sprayed tef (Bayeh et al., 2009).

In spite of the economic implications of tef shoot fly damage on tef in Tigray region particularly in Mekoni, Axum and Wukro districts (DZARC, 2002; Bayeh et al.,

2008), development of host plant resistance were not attempted. Host plant resistance can play a major role in minimizing the extent of losses and is compatible with other tactics of pest management, including the use of natural enemies and chemical control (Kumar et al., 2008). Therefore, having the diversity of tef in Ethiopia and evaluating them for the reaction tef shoot fly can lead to get a material host plant resistance. The main objective of this study was to assess the genetic variation among tef genotypes for the reaction to tef shoot fly.

MATERIALS AND METHODS

Description of the study area

The field experiment was carried out at Axum Agricultural Research Center (AxARC) during 2015 main cropping season at the substation Maysiye (14° 6'43" North and 38° 36'41" East, altitude of 2200 masl) in Tahitaey Maichew district, in central zone of Tigray, Ethiopia. The substation is located at 17 km west of Axum town. The annual rainfall received by the experimental site during the main cropping season was 613.92 mm. Moreover, the mean average annual minimum and maximum temperature was 12.16 and 26.78°C, respectively.

The experimental material consisted of 49 genotypes of which 32 released tef varieties, 12 promising lines, three accessions collected from tigray region and two local landraces obtained from Deber Zeit Agricultural Research Center (DZARC), Axum Agriculture Research Centers and farmers, respectively used as standard checks. The tef seeds were sown in the third week of July, 2015.

Experimental design and management

The experiment was laid out in 7×7 triple lattice designs. Each tef seeds from each genotype was sown in three rows of 2 m length spacing at 0.2 m inter row spacing plots, blocks and replications were spaced at 1, 0.5 and 1.5 m, respectively.

In accordance with the recommended tef seed rate of 10 kg/ha (AxARC, 2013/2014), 1.2 g of seeds per plot was hand-drilled in the rows. Fertilizer rates of 60 kg N and 40 kg P₂O₅ ha⁻¹ was used (Seyfu, 1997). The source of nitrogen was urea and di-ammonium phosphate (DAP) was the source of phosphorus. DAP was applied once at the time of sowing, while urea was applied in split after germination. The first urea application was made two weeks after seed germination and the second split was applied two weeks later after the first application.

All other cultural crop management practices were applied as per the recommendation for tef production. To ensure uniform distribution of tef shoot fly infestation, fish meal (dried and powdered fish meal) was broadcasted at a rate of 15 g/plot in two phases on all entries. The first fish meal application was made at the first appearance of the tef shoot fly damage symptom in early vegetative stage and the second was made during heading stage. As reported by Jotwani and Young (1972), the fish meal was used to ensure the infestation on sorghum shoot fly resistance materials.

Data collection

The number of infested plants was estimated by counting the plants with dead heart at the interval of three days starting from the first appearance of damage symptom. At each count, the infested plant

was tagged with thread. White heads were counted or noted at pre harvesting (physiological) maturity. The numbers of infested and non-infested productive tillers were also counted from randomly selected ten plants. At harvesting, total population were counted (including tillers raised from infested and non-infested plants) and the sum of dead heart plus the white head per plot used for determining percent damage.

$$\text{Dead heart (\%)} = \frac{\text{Total number of dead hearts}}{\text{Total number of productive tillers}} \times 100$$

$$\text{White head (\%)} = \frac{\text{Total number of whitehead}}{\text{Total number of productive tillers}} \times 100$$

$$\text{Damage (\%)} = \frac{\text{Total number of dead hearts + white heads}}{\text{Total number of productive tillers}} \times 100$$

The actual mean of dead heart plus white headed percentage used for classification of the tef genotypes under different categories. This categorizing way was taken by adopting the rating scale of the International Rice Research Institute (IRRI), viz. Standard Evaluation System (SES) for rice (Table 2) (Visalakshmi et al., 2014).

Grain yield was measured from infested and non infested plants each from ten main plants with their tillers. The plants were hand threshed, weighed and the weight was divided by the total number of productive tillers of the ten sample plants. The grain yield data from infested and non-infested plants was used for yield loss estimation. Yield loss due to the tef shoot fly damage was estimated using the analytical methods of yield loss as stated by Judenko (1972) formula:

ACT = Actual yield (e.g. grain yield per plot)

a = Mean yield per uninfested plant

b = Mean yield per infested plant

p = Percentage of plants infested

1. The coefficient of harmfulness (C) is

$$C = \frac{(a - b)100}{a}$$

2. Percentage economic loss (L) is

$$L = \frac{CP}{100}$$

3. Expected yield in the absence of the pest (W) is

$$W = \frac{100(ACT)}{100 - L}$$

4. The economic loss (LOS) is

$$LOS = W - ACT$$

RESULTS AND DISCUSSION

Reaction of tef genotypes to the tef shoot fly infestation is

presented in Table 1. Based on the damage (%) of dead heart plus white heads, only one accession viz. acc. 17 WJ was grouped under resistant category based on the damage of dead heart plus white heads. In general, 10, 19, 16, and 3 tef genotypes were grouped as moderately resistant, moderately susceptible, susceptible and highly susceptible to tef shoot fly attach, respectively. As plant resistant to insect pest is known to be due to antibiosis, tolerance and antixenosis (Abro et al., 2003), the mechanism of resistance observed for 17 WJ in the current study requires further investigation in order to reach at concrete conclusion.

The tef shoot fly infestation started from two weeks after emergence up to the end of heading. This trial was sown on 22 July, 2015 and the shoot fly infestation started on 10 August, 2015 when the seedlings reached three leaf stages. The infestation continued up to September 4, 2015. The pattern of tef shoot fly infestation was less at early seedling stage, it slowly increased as the season progresses and finally become slow. Mostly, the shoot fly affects seedling and newly raised tillers and in few intensity at heading. DZARC (1983) reported late sown tef is infested by the tef shoot fly, while early sown tef is infested only if there is dry spell.

Conditions like late sowing date, poor germination and production of tillers at later stage increased the level of infestation. During the crop growth sparsely populated varieties compensated for lost plants by producing more tillers. However, late produced tillers were severely affected by tef shoot fly. Some tef varieties such as Melko might be inherently susceptible to tef shoot fly and as a result they had high level of infestation. Moreover, use of yield enhancing inputs like chemical fertilizer and manure might also increases the infestation of tef shoot fly. Corbeels et al. (2000) and Berhane et al. (2015) had similar observation on the effect of inputs for tef production that stimulates the appearance of shoot fly. In general, dead hearts were more prevalent in fertile parts than in the waterlogged or less fertile parts of the tef field (Tesfaye and Zenebe, 1998). This input increase the infestation due to making the plant softy favorable for food and to lay egg.

The damage due to tef shoot fly ranged from 4.21% from acc.17 WJ to 36.92% for Melko per plot. The results are in agreement with the previous reports of 6.96 to 37.60% damage in tef due to tef shoot fly infestation in Tigray regional state (DZARC, 2002). The standard checks like Quncho and Kora have been grouped under the susceptible category. However, the local check was categorized with moderately susceptible. Three varieties, namely, Melko, Gerado and Koye had the highest damage rate. The majority of the genotypes were classified as moderately susceptible and susceptible.

Tef shoot fly is becoming economic important insect pest in the last three to five years in the study area. The estimated yield loss for genotypes had positive and negative sign (Table 1). The positive value indicates

Table 1. Reaction of tef genotypes to tef shoot fly damage and associated yield loss at Myseiye in 2015.

S/N	Genotypes	Tef shoot fly damage (percentage of dead heart)	Tef shoot fly damage (percentage of white headed)	Tef shoot fly damage (percentage of dead heart and white headed %)	Damage rate (scale)	Resistance category	Yield loss (kg ha^{-1})	Gy (kg ha^{-1})
1	DZ-01-99 (Asgori)	11.80	0.8514	12.65	5	MS	-88.08	2335.60 ^a
2	DZ-01-354 (Enatit)	15.03	0.8007	15.83	7	S	81.58	2298.10 ^a
3	DZ-01-196 (Magna)	14.70	0.9346	15.63	7	S	-15.92	2064.70 ^{ab}
4	DZ-01-787 (Wellenkomi)	14.91	0.5098	15.42	5	MS	-8.00	1832.50 ^{ab}
5	DZ-Cr-44 (Menagesha)	11.90	0.9863	12.89	5	MS	58.42	2255.80 ^a
6	DZ-Cr-82 (Melko)	35.14	1.7791	36.92	9	HS	-168.50	1385.80 ^{ab}
7	DZ-Cr-37(Tsedey)	6.86	0.3328	7.19	3	MR	-9.92	2085.60 ^{ab}
8	DZ-Cr-255 (Gibe)	12.66	0.6846	13.34	5	MS	-13.83	2286.70 ^a
9	DZ-Cr-358 (Ziquala)	22.30	0.4321	22.73	7	S	-15.25	1854.20 ^{ab}
10	DZ-01-974 (Dukem)	16.81	0.3939	17.20	7	S	-160.50	1475.80 ^{ab}
11	DZ-01-1281(Gerado)	28.17	1.2874	29.46	7	HS	3.58	1761.20 ^{ab}
12	DZ-01-1285 (Koye)	25.49	1.7600	27.25	9	HS	-84.83	1667.20 ^{ab}
13	DZ-01-1681 (Key Tena)	9.65	0.4508	10.10	3	MR	-3.08	2160.80 ^{ab}
14	DZ-01-899 (Gimbichu)	13.22	0.4680	13.69	5	MS	18.42	1882.30 ^{ab}
15	DZ-01-2675 (DegaTef)	22.69	1.5704	24.26	7	S	-231.83	1908.50 ^{ab}
16	DZ-Cr-387RIL355 (Quncho)	16.14	0.7524	16.89	7	S	-13.13	2130.80 ^{ab}
17	Ho-Cr-136 (Amarach)	7.71	0.8393	8.55	3	MR	-6.50	2177.20 ^a
18	DZ-Cr-285 RIL295 (Simada)	6.51	0.7089	7.22	3	MR	22.75	2139.20 ^{ab}
19	DZ-01-2053 (Holetta Key)	21.21	0.9094	22.12	7	S	-89.75	1011.10 ^b
20	DZ-01-1278 (Ambo Toke)	16.94	0.6704	17.61	7	S	-169.33	1874.40 ^{ab}
21	9441	11.39	0.6255	12.02	5	MS	-14.50	2109.70 ^{ab}
22	DZ-01-2054 (Gola)	14.66	0.7554	15.42	5	MS	-70.58	2091.70 ^{ab}
23	DZ-01-146 (Genete)	12.37	0.8558	13.23	5	MS	-41.33	1917.90 ^{ab}
24	DZ-01-1821 (Zobel)	12.14	0.9754	13.12	5	MS	11.58	2081.10 ^{ab}
25	Acc. 205953 (Mechare)	12.83	0.6863	13.52	5	MS	47.08	2306.90 ^a
26	RIL273 (Laketch)	6.73	0.4570	7.19	3	MR	39.92	1555.80 ^{ab}
27	DZ-01-1868 (Yilmana)	17.70	1.0793	18.78	7	S	-190.92	2337.90 ^a
28	DZ-01-2423 (Dima)	7.09	0.4093	7.50	3	MR	-47.75	2129.70 ^{ab}
29	DZ-01-3186 (Etsub)	15.79	0.6023	16.39	7	MS	49.33	1569.20 ^{ab}
30	DZ-01-1880 (Guduru)	13.96	1.1588	15.12	5	MS	90.33	2377.20 ^a
31	acc. 17 WJ	3.76	0.4497	4.21	1	R	-20.08	2278.90 ^a
32	PGRC/E 205396 (Ajora)	10.46	1.4075	11.87	5	MS	-151.00	1633.30 ^{ab}
33	DZ-Cr-409/RIL50d (Boset)	14.84	0.9261	15.77	7	S	-104.33	1797.90 ^{ab}
34	Kora	22.02	1.1533	23.17	7	S	-36.92	-
35	Zagre (local 1)	7.65	0.4812	8.13	3	MR	-41.33	2017.50 ^{ab}

Table 1. Contd.

36	Zezew (local-2)	4.09	1.6304	5.72	3	MR	-19.58	2093.10 ^{ab}
37	acc. 13 –AI	6.35	1.1048	7.45	3	MR	-24.92	2094.70 ^{ab}
38	RIL -65	11.18	1.2193	12.40	5	MS	30.33	1999.40 ^{ab}
39	RIL-86	10.66	0.9183	11.58	3	MR	26.67	2046.70 ^{ab}
40	RIL-190	23.24	0.7218	23.96	7	S	66.83	1816.90 ^{ab}
41	RIL-96	17.34	1.1036	18.44	7	S	20.75	1629.70 ^{ab}
42	RIL-109A	13.92	0.7699	14.69	5	MS	-185.83	2151.10 ^{ab}
43	RIL-15A	13.80	0.8930	14.69	5	MS	-108.42	1573.60 ^{ab}
44	RIL-52	14.42	0.1544	14.57	5	MS	-7.25	1665.80 ^{ab}
45	RIL-129A	10.92	1.2133	12.13	5	MS	-13.75	1541.10 ^{ab}
46	RIL-91Ap	18.92	0.5273	19.45	7	S	17.25	1659.60 ^{ab}
47	RIL-101C	21.26	0.4954	21.76	7	S	-43.08	1746.10 ^{ab}
48	RIL-107	15.93	0.3889	16.32	7	S	97.83	1841.70 ^{ab}
49	RIL157	9.65	1.2612	10.91	5	MS	5.08	2125.00 ^{ab}

R=resistance, MR=moderately resistance, MS=moderately susceptible, S=susceptible, HS=highly susceptible.

Table 2. Percentage damage and rating scale used to determine the level of tef resistance to tef shoot fly.

Damage (%)	Rating scale	Degree of resistance
0	0	Highly resistance (HR)
1-5	1	Resistance (R)
6-10	3	Moderately resistance (MR)
11-15	5	Moderately susceptible (MS)
16-25	7	Susceptible (S)
>26	9	Highly susceptible (HS)

actual yield loss due the tef shoot fly infestation. Whereas, the negative value indicates yield from shoot fly infested plants was greater than the yield from non-infested plants. Compensation of lost parts is one mechanism of resistance to insect pests.

The yield loss ranged from 3.58 for Gerado to 97.83 kg ha⁻¹ for RIL-107. The yield loss had a

negative and positive sign, which indicates the loss yield due to the infestation had positive sign, whereas the positive sign showed that the infestation of the fly on the tef plant can increase the yield over the un-infested plant. Therefore, some of tef genotypes increased their yield due to tef shoot fly infestation. The yield increased due to tef shoot fly infestation which was minimum, 3.08

kg ha⁻¹ for Key Ten and maximum, 231.83 kg ha⁻¹ for Dega Tef. Insects like shoot flies are known to encourage more tiller production at low level of infestation and when moisture is not limiting. Even though, it stimulates grain yield on the infested individual, it does not enhance the genetic make of the tef genotypes. However, these genotypes which were over-compensatory would be used for

breeding programme.

On the bases of yield loss, 16 and 3 genotypes were susceptible and highly susceptible, respectively. Susceptible indicates because they were not able to compensate the damaged parts by the tef shoot fly. However, at Haramaya (Alemaya) tef is sown after sorghum and the shoot fly population that built-up on sorghum might have caused severe damage (378 to 522 kg ha⁻¹) (Sileshi, 1997).

As far as the yield loss due to tef shoot fly is concerned, out of the total ten moderately resistant tef genotypes, only Simada and lakech had positive yield loss, implying that there is an actual yield loss due to this particular insect pest. Despite they had low damage percent; the yield loss indicated that these genotypes were susceptible.

On the contrary, out of 19 moderate susceptible genotypes, only eight genotypes showed positive yield loss. However, the remaining genotypes showed negative yield loss. Therefore, these genotypes were not grouped under the susceptible category due to the yield gain.

In general, the evaluation based on the damage percent alone cannot give full information on resistance and susceptibility of the tef genotypes to tef shoot fly. Therefore, looking on both damage percent and yield loss would be the accurate method for the categorization of genotypes. Genotypes having resistance and moderately resistance genotypes would better to have at least 0 to negative yield loss. Whereas, moderately susceptible, susceptible and highly susceptible genotypes by the damage percent also must have yield loss value of greater than 0.

In general, in tef growing areas, with moisture limitation tef shoot fly infestation causes yield losses. Therefore, providing resistant variety (acc.17 WJ) and chemical application is recommended for these areas.

CONCLUSION AND RECOMMENDATIONS

The acc. 17 WJ was a resistance for tef shoot fly as the result revealed. While, the standard checks were categorized or grouped under the susceptible category. Therefore, the acc. 17 WJ is recommended for the tef shoot fly resistance. Tef damage (dead heart and white headed) due to tef shoot fly ranged from 4.20 to 36.96%. Consequently, the yield loss also varied from 3.58 to 97.83 kg ha⁻¹. Although tef shoot fly infestation caused yield losses in some genotypes, it had increased grain yield in some other tef genotypes. The evaluated 49 tef genotypes were classified into resistant, moderately resistant, moderately susceptible, and susceptible and highly susceptible (Table 1).

Duration of tef shoot fly infestation determined between the beginnings of August to the end of September. Conditions favorable to tef shoot fly are the erratic rainfall, inherently susceptibility of tef genotypes, late

produced productive tillers and inputs (chemical and manure fertilizers), which are good environment for the reproduction and growth of the fly. Therefore, the genotypes which exhibited resistant and moderately resistant tef shoot fly damage would be important for the production of tef. In areas where the mentioned condition is faced and the infestation of shoot fly occurred, the application of chemical at early stage is recommended. Moreover, evaluation of the tef genotypes at multi-location for further investigation is better.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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