Selection of extra-early maize inbreds under low N and drought at flowering and grain-filling for hybrid production

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Keywords: drought stress, genotype x trait biplot, low-N tolerance, multiple trait selection, extra-early maize

Abstract
Extra-early maize (Zea mays L) tolerant to low-N and drought could stabilize yields in sub-Saharan Africa. Studies were conducted under drought and low-N conditions in Nigeria for three years to determine the potential of 90 extra-early inbreds for hybrid production and evaluate the performance of 36 hybrids derived from the inbreds under drought at flowering and grain-filling periods and well-watered conditions. Results showed significant genotypic mean squares for grain yield and most other traits of the inbreds under drought and/or low-N conditions. TZEEI6, TZEEI4, TZEEI36, and TZEEI38 were identified as ideal inbreds under drought. Under low-N, TZEEI19, TZEEI96 and TZEEI45 were top ranking with TZEEI19 as the ideal inbred. TZEEI19, TZEEI29, TZEEI56, TZEEI38, and TZEEI79 were tolerant to both stresses. Eighteen of the 36 hybrids produced above-average yields across environments with four hybrids identified as very stable. TZEEI29 x TZEEI21 was the closest to the ideal genotype because it combines large mean performance with high yield stability. Hybrid yield under drought had large positive correlation with grain yield under well-watered environments. Selection for inbred traits such as days to silking and anthesis-silking interval under drought predicted fairly accurately hybrid yield under well-watered environments. It was concluded that extra-early inbreds and hybrids are not only drought escaping but also possess drought and low-N tolerant genes.

Keyword: drought stress, genotype x trait biplot, low-N tolerance, multiple trait selection, extra-early maize

Introduction
Maize is the most important cereal crop after rice in West and Central Africa (WCA). However, during the last two decades, production and productivity have lagged behind population growth for several reasons, including low soil fertility; little or no use of improved seeds, herbicides, and fertilizers; inadequate plant density, weed infestation, poor tillage practices, labour shortages, increased levels of biotic and abiotic constraints, and high costs of inputs. In addition, serious infrastructural and institutional constraints have limited the adoption of improved maize technologies. Global warming and its associated effects have changed weather patterns leading to erratic and unreliable amount and distribution of rainfall, resulting in drought. Presently, stresses from Striga infestation, drought, and low N constitute the most important biotic and abiotic factors limiting maize production in WCA.

A major strategy of the Maize Improvement Program at the International Institute of Tropical Agriculture (IITA) is to breed for Striga resistant, drought and low-N tolerant maize cultivars to increase and stabilize maize yield production in the sub-region. Two approaches have been adopted for drought tolerance. The first is to breed for extra-early maturing cultivars that are drought escaping since they mature before the onset of terminal drought. The second strategy is to introgress into the early and extra-early cultivars genes for drought tolerance to enable them withstand mid-season drought when it occurs during the flowering and grain-filling periods.

In most of the developing countries of the world, maize production is carried out under conditions of low soil fertility (Mccown et al., 1992; Oikeh and Horst, 2001). The inherent low-N status of soils in the savannas of WCA, coupled with the little or no use of N-fertilizers in the region further compounds the problem of drought stress and Striga infestation on maize productivity. Estimated yield losses from N stress alone can be as high as 50% (Wolfe et al., 1988). Therefore, the development and adoption of maize germplasm with tolerance to multiple stresses are crucial for increased productivity. Bänziger et al. (1999) showed that improvement for drought tolerance also resulted in specific adaptation and improved performance under low-N conditions, suggesting that tolerance to either stress involves a common adaptive mechanism. Due to low heritability under stress conditions (Badu-Apraku et al., 2004, 2005), the use of secondary traits has been proposed for yield improvement. Secondary traits such as ears per plant (EPP), rate

Maydica 56-1721
of leaf senescence (LS), and anthesis-silking interval (ASI) have strong associations with yield under low-N and drought conditions and have been used to select for higher levels of tolerance to the two stresses in maize (Lafitte and Edmeades, 1995; Bänziger and Lafitte, 1997).

During the last two decades, IITA in collaboration with the National Agricultural Research Systems (NARS) of WCA has developed a wide range of high yielding, drought tolerant or escaping early and extra-early populations (white and yellow endosperm), inbred lines, and cultivars in an effort to combat the threat posed by recurrent drought and low N in the savannas of WCA. The extra-early populations from which the inbred lines and cultivars were derived were formed from crosses between local (landraces) with exotic and introduced germplasm identified through extensive multilocation trials in WCA (Badu-Apraku and Fakorede, 2001; Badu-Apraku et al., 2007). These materials were selected on the basis of high grain yield, earliness, resistance to the maize streak virus and, above all, adaptation to the heat and drought stresses characteristic of the Sudan savannas in Burkina Faso, Mali, Mauritania, Ghana, and Nigeria during both the rainy and dry seasons. It was hypothesized that, after several years of cultivation in these environments, the extra-early germplasm should have adaptive traits for tolerance to these stresses where they had survived. We observed that some of the extra-early inbred lines in the IITA Maize Program would not only escape drought stress but also seemed to possess drought tolerance genes. The inbreds should therefore be able to withstand the mid-season drought that occurs during the flowering and grain-filling periods in the savannas of WCA as have been found in early, intermediate, and late maturing cultivars.

The breeder is constrained by a number of problems while attempting to use secondary traits as indirect selection criteria for yield improvement. Prominent among the problems is identification of the most appropriate traits and the genotypes demonstrating high expressivity and heritability for the traits. Use of multiple regression and multivariate techniques have been proposed, but these are of limited applicability in this regard (Badu-Apraku and Akinwale, 2011). Yan and Rajcan (2002) proposed the genotype-by-trait (GT) biplot for analysis of multiple trait data. The method graphically displays the genotype-by-trait table and permits the associations among traits and the trait profile of the genotypes to be visualized across the genotypes. This method appears to be a potent tool for effectively identifying traits to be used as indirect selection criteria as well as genotypes possessing such traits. A new maize cultivar must possess the minimum criterion established by the objectives of the breeding program as well as other desirable agronomic and end user-preferred traits which may be measured by selection index. If the genotype fails to meet the minimum criterion for any breeding objective, this will result in non-adoption of the genotype by farmers and consumers. Under such circumstances, the independent culling and index selection have to be adopted (Yan and Fregeau-Reid, 2008). Therefore, Yan and Fregeau-Reid (2008) proposed the comprehensive selection scheme for considering all breeding objectives and their relative importance in the ideal cultivar. The scheme consists of three strategies: (i) independent selection, in which different traits are selected in different generations; (ii) independent culling, involving multiple trait selection simultaneously (concurrently) and independently; and (iii) index selection in which multiple traits are selected simultaneously by an index that is a linear combination of various traits, with traits treated as compensational (Yan and Fregeau-Reid, 2008). The objectives of this study were to (i) screen extra-early inbred lines in the IITA Maize Program for tolerance to low-N and drought stress imposed at flowering and grain-filling periods (ii) identify low-N and drought tolerant extra-early inbreds as candidate parents with favorable alleles for introgression into maize breeding populations and for the production of drought tolerant extra-early hybrids, and (iii) predict hybrid performance from inbred line traits under drought stress.

Materials and Methods

Germplasm

Ninety extra-early inbred lines developed from five broad-based populations resistant to Striga and maize streak virus and the 36 single-cross hybrids developed from selected drought tolerant inbreds among the 90 inbreds in our Program were used in the three studies reported in this paper. The inbred lines were derived from the two broad-based populations, TZEE-W Pop STR C_5 and TZEE-Y Pop STR C_6 and the crosses, TZEE-W SR BC5 x 1368 STR, TZEE-W Pop STR x LD and TZEF-Y SR BC1 x 9450 STR. The two populations, TZEE-W Pop STR C_5 and TZEE-Y Pop STR C_6 were formed from local and exotic germplasm identified through several years of extensive testing in WCA (Badu-Apraku et al., 2007). The method adopted for the development of the inbred lines has been described in detail by Badu-Apraku and Menkir (2006). The lines were developed by the pedigree method with evaluation of topcross performance at the S_0 stage under Striga infestation at Ferkesedougou (9°3’N, 5°10’W, mean annual rainfall of 1,400 mm) and in Striga-free conditions at Sinematiali (9°37’N, 3°04’W, mean annual rainfall of 1,200 mm) both in Côte d’Ivoire, during the rainy seasons of 1997 and 1998. For the topcrosses, 250–300 lines derived from each population were crossed to the corresponding base population as the tester as proposed by Hallauer and Miranda (1988). There was no deliberate selection for drought tolerance. The yield performance of the lines per se, their combining
Table 1 - Relative trait values (0,1), selection index, and decisions made on independent selection, independent culling, and index selection for 22 selected inbreds under drought stress at Ikenne and 15 selected inbreds under low-N conditions at Mokwa out of 90 extra-early maturing maize inbred lines evaluated in Nigeria between 2007 and 2009.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Grain yield</th>
<th>Plant aspect</th>
<th>Ears per plant</th>
<th>Ear aspect</th>
<th>Anthesis-silking interval at 56 DAP</th>
<th>Leaf senescence at 70 DAP</th>
<th>Selection Index</th>
<th>Selected fort</th>
<th>Culled fort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drought stress environments</td>
<td>Weight</td>
<td>1.00</td>
<td>-0.50</td>
<td>0.50</td>
<td>-0.50</td>
<td>-0.50</td>
<td>-0.50</td>
<td>1.00</td>
<td>YD_H</td>
</tr>
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<td>TZEE16</td>
<td>0.90</td>
<td>0.10</td>
<td>0.85</td>
<td>0.06</td>
<td>0.21</td>
<td>0.00</td>
<td>0.11</td>
<td>0.38</td>
<td>YD_H</td>
</tr>
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<td>0.33</td>
<td>0.66</td>
<td>0.00</td>
<td>0.43</td>
<td>0.00</td>
<td>0.38</td>
<td>0.38</td>
<td>EPP_H</td>
</tr>
<tr>
<td>TZEE14</td>
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<td>0.17</td>
<td>0.54</td>
<td>0.00</td>
<td>0.10</td>
<td>0.00</td>
<td>0.00</td>
<td>0.65</td>
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</tr>
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<td>0.11</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.65</td>
<td>EPP_H</td>
</tr>
<tr>
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<td>0.90</td>
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<td>1.00</td>
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<td>0.43</td>
<td>0.00</td>
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<td>0.07</td>
<td>0.74</td>
<td>0.26</td>
<td>0.00</td>
<td>0.43</td>
<td>0.00</td>
<td>0.38</td>
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</tr>
<tr>
<td>TZEE6</td>
<td>0.54</td>
<td>0.00</td>
<td>0.75</td>
<td>0.13</td>
<td>0.26</td>
<td>0.00</td>
<td>0.00</td>
<td>0.38</td>
<td>EPP_H</td>
</tr>
</tbody>
</table>

| Low N environments | Weight | 1.00 | -0.50 | 0.50 | -0.50 | -0.50 | -0.25 | -0.25 |
|TZEE145 | 0.89 | 0.00 | 0.17 | 0.00 | 0.47 | 0.00 | 0.00 | 0.00 |
|TZEE119 | 0.75 | 0.10 | 0.18 | 0.00 | 0.41 | 0.00 | 0.00 | 0.00 |
|TZEE140 | 1.00 | 0.28 | 0.14 | 0.21 | 0.47 | 0.00 | 0.00 | 0.00 |
|TZEE29 | 0.61 | 0.14 | 0.17 | 0.00 | 0.53 | 0.00 | 0.00 | 0.00 |
|TZEE96 | 0.38 | 0.17 | 0.19 | 0.00 | 0.41 | 0.00 | 0.00 | 0.00 |
|TZEE79 | 0.56 | 0.17 | 0.16 | 0.13 | 0.47 | 0.00 | 0.00 | 0.00 |
|TZEE22 | 0.66 | 0.28 | 0.21 | 0.17 | 0.47 | 0.00 | 0.00 | 0.00 |
|TZEE65 | 0.41 | 0.03 | 0.15 | 0.08 | 0.47 | 0.00 | 0.00 | 0.00 |
|TZEE82 | 0.58 | 0.17 | 0.18 | 0.21 | 0.47 | 0.00 | 0.00 | 0.00 |
|TZEE54 | 0.47 | 0.17 | 0.17 | 0.53 | 0.18 | 0.00 | 0.00 | 0.00 |
|TZEE85 | 0.45 | 0.07 | 0.16 | 0.08 | 0.47 | 0.18 | 0.00 | 0.00 |
|TZEE24 | 0.47 | 0.00 | 0.18 | 0.38 | 0.47 | 0.18 | 0.00 | 0.00 |
|TZEE36 | 0.58 | 0.24 | 0.19 | 0.08 | 0.36 | 0.18 | 0.00 | 0.00 |
|TZEE38 | 0.37 | 0.24 | 0.14 | 0.25 | 0.00 | 0.46 | 0.18 | 0.00 |
|TZEE90 | 0.48 | 0.28 | 0.14 | 0.00 | 0.53 | 0.64 | 0.46 | 0.00 |

¶ H= Selected for high levels and L=Selected for low levels of the relevant traits; † H=Culled for high levels and L=Culled for low levels of the relevant traits.

Field evaluations

Three experiments were conducted between 2007 and 2010 in Nigeria to identify extra-early inbreds for tolerance to low N and/or drought stress at the flowering and grain-filling periods, and to determine the potential of the inbreds for hybrid production and as source germplasm for the improvement of breeding populations. In the first two experiments, the 90 extra-early maturing maize inbred lines were evaluated at Ikenne (6º53’N, 3º42’E, 60 m altitude, 1200 mm annual rainfall) in Nigeria under managed drought stress and in well-watered environments during the dry seasons of 2007/2008 and 2008/2009. Similarly, the lines were evaluated in low-N (30 kg ha⁻¹) and high-N (90 kg ha⁻¹) studies at Mokwa (9º18’N, 5º4’E, 457 m altitude, 1,100 mm annual rainfall) during the planting seasons of 2008 and 2009. A randomized incomplete block design (9 x 10 alpha lattice) with two replications was used for all inbred trials. Each experimental unit was a one-row plot, 5 m long with a row spacing of 0.75 m. The hybrids were evaluated using 6 x 6 simple lattice design. Distance between two adjacent plants within the row was 0.40 m in all trials. Three seeds were planted per hill and the seedlings were thinned to two per hill about 2 weeks after emergence to give a final plant population density of about 66,000 plants ha⁻¹.

Induced drought stress at Ikenne was achieved by withdrawing irrigation water from 21 days after planting (DAP) until maturity so that the maize plants relied on stored water in the soil for growth and development. During the first three weeks...
of growth, the plants were irrigated using a sprinkler irrigation system which provided 12 mm of water per week. The soil in the experiment station at Ikenne is Alfisol (United States Soil Taxonomy classification) and the experimental fields at the station are flat and fairly uniform, with high water-holding capacity. The well-watered (rainfed) experiment at Ikenne was conducted during the growing season. Except for the well-watered treatments, all management practices were the same for both well-watered and drought stress studies. Fertilizer was applied to the well-watered and drought stress plots at the rate of 60 kg ha\(^{-1}\) each of N, P and K at planting. An additional 60 kg ha\(^{-1}\) N was top-dressed at 2 WAP. The trials were kept weed-free with the application of Atrazine and Gramoxone as pre- and post-emergence herbicides at 5 L/ha each of Primextra and Paraquat, and subsequently, by hand weeding.

In the second experiment conducted at Mokwa in 2008 and 2009, the 90 extra-early maturing maize inbred lines used for the first experiment were evaluated in experimental fields which had been depleted of N by continuously planting maize and removing the biomass after each harvest for five years. The soil at Mokwa is an Alfisol with 0.27% organic carbon content, 0.035% organic nitrogen, and 0.48% phosphorus content. Soil samples were taken each year before planting for all the test environments and N content was determined at the IITA's analytical services laboratory at Ibadan. The total N in the soils was determined by Kjeldahl digestion and colorimetric determination on Technicon AAII Autoanalyser (Bremner and Mulvaney, 1982). Fertilizers were applied to bring the total available N to 90 kg ha\(^{-1}\) for the field

![Figure 1](image)

Figure 1 - A vector view of genotype-by-trait biplot showing the ranking of the 90 inbreds on the basis of their mean performance across six selected drought tolerance traits. The biplot was based on trait-focused singular value partitioning (‘SVP = 1’) and is therefore appropriate for visualizing the relationships among genotype. Principal component PC1 and PC2 for model 2 explained 63.6% of the variation among traits. ‘*’ followed by a number represents inbred line that were closest to the ideal genotype under drought stress. (*) represents exact positions of other inbreds that were less responsive under drought stress.)
with moderately high-N treatment, and 30 kg ha\(^{-1}\) for the low N as indicated by soil tests. The N-fertilizer was applied at two weeks after planting (WAP) immediately after thinning. Also, single superphosphate (\(\text{P}_2\text{O}_5\)) and muriate of potash (\(\text{K}_2\text{O}\)) were applied to both low-N and high-N blocks at the rate of 60 kg ha\(^{-1}\). Other crop management practices were as described earlier for the first study.

In the third experiment, 36 single cross hybrids formed from lines selected from the 90 inbreds for tolerance to managed drought stress at the flowering and grain-filling periods were evaluated under natural drought stress at Bagauda and in well-watered conditions (rainfed) at Ikenne and Zaria during the growing season of 2009 and under managed drought stress at Ikenne during the 2009/2010 dry season. The inbred parents were selected based on their superior yield performance under drought stress. The experimental design and management practices for both drought stress and well-watered studies were the same as described for the first experiment except that there were two-row plots per treatment.

**Collection of field data**

Data were recorded in all experiments for days to 50% silking (DS) and anthesis (DA), as the number of days from planting to when 50% of the plants had silks emerged and shed pollen, respectively. The anthesis-silking interval (ASI) was calculated as the difference between DS and DA. Plant height (PH) was measured as the distance from the base of the plant to the height of the first tassel branch and ear height (EH), as the distance to the node bearing the top ear. Root lodging (RL) (percentage of plants leaning more than 30º from the vertical), stalk lodging (SL) (percentage broken at or below the highest ear node), and disease reaction were also recorded. Ear number per plant (EPP) was obtained by dividing the total number of ears per plot by the number of plants harvested. Plant aspect (PA) was recorded on a scale of 1 to 5 based on plant type, where 1 = excellent and 5 = poor. Husk cover (HC) was rated on a scale of 1 to 5, where 1 = husks tightly arranged and extended beyond the ear tip and 5 = ear tips exposed. Ear aspect (EA) was based on a scale of 1 to 5, where 1 = clean, uniform, large, and well-filled ears and 5 = ears with undesirable features. In addition, leaf death scores (LS) were recorded for the drought stress experiments at 70 DAP and for low-N plots at 56 (LS1) and 70 (LS2) DAP on a scale of 1 to 9, where 1 = almost all leaves green and 9 = virtually all leaves dead. In the drought stress and low-N experiments, harvested ears from each plot were shelled to determine the percentage grain moisture. Grain yield was adjusted to 15% moisture and computed from the shelled grain weight. On the other hand, in the well-watered and high-N experiments, harvested ears of each plot were weighed and grain yield (YD) was computed based on 80% (800 g grain kg\(^{-1}\) ear weight) shelling percentage before adjusting moisture content.

**Data analysis**

Analyses of variance (ANOVA), combined across environments were performed on plot means for grain yield, DS, ASI, EPP, PH, percentage SL, leaf death score, EA, PA, and HC with PROC GLM in SAS (SAS Institute, 2001). ANOVA was performed separately for data collected from drought stress and well-watered plots, the low-N and high-N environments for the selected traits of the inbreds in the first and second studies and for the single-cross hybrids in the third study. In the combined ANOVA, the location/year combinations, replicates, and blocks of each experiment were considered as random factors while entries were considered as fixed effects.

Phenotypic and genotypic correlations between pairs of agronomic traits and their standard errors were computed with the restricted maximum likelihood (REML) method (Holland, 2006) using Proc MIXED of the SAS system (SAS Institute, 1999). A phenotypic or genetic correlation was considered significant when its absolute value was larger than twice its standard error. In addition, regression analyses were performed to assess the interrelationship among inbred per se and hybrid performance under drought stress and well-watered environments, using mid-parent values of inbred Per se traits as the independent variables and the corresponding hybrid yield as the dependent variable.

Analysis of variance was also carried out for each environment (location/year combinations) and also across all environments for each of the experiments to determine if genotype × environment (G × E) interaction was significant. GGE biplot software was employed for trait-association and trait-profile analyses (Yan et al, 2000; Yan, 2001; Yan and Rajcan, 2002; Morris et al, 2004; Ober et al, 2005). Since the traits were measured in different units, the data were standardized using standard deviation method (“Scale = 1”), not transformed (“Transform = 0”) and trait-centered (“Centering = 2”). Therefore, the outputs are appropriate for visualizing the relationships among genotype and traits. The vector (“mean-vs-stability”) view of the GT biplot were based on the six selected traits (YD, EPP, PA, EA, ASI and LS) used in the base index for selecting for drought and low-N tolerance in the IITA Maize Program. Equal weight was attached to the selected traits and the traits PA, EA, ASI, and LS that are often negatively correlated to YD and EPP were re-scaled such that large values for all the traits were desirable. This made all the selected traits to fall on one side of the ATC ordinate. These conditions were required to identify superior and ideal genotypes based on the “mean-vs-stability” view of the GGE biplot (Yan et al, 2007). The polygon views (or “which-won-where” view of the biplot were based on genotype-focused singular value partitioning (“SVP = 2”) and were therefore appropriate for visualizing
Figure 2 - A vector view of genotype-by-trait biplot showing the ranking of 90 inbreds under low-N conditions on the basis of their mean performance across selected traits. The biplot was based on trait-focused singular value partitioning (SVP = 1) and is therefore appropriate for visualizing the relationships among traits. Principal component PC1 and PC2 for model 2 explained 70.8% of the variation among genotype. 'T' followed by a number represents inbred lines that were closest to the ideal genotype under low N conditions. (*) represents the exact positions of other inbreds that were less responsive under low N).

the relationships among traits while the vector views were based on the environment-focused singular value partitioning (SVP = 1) and were therefore appropriate for visualizing the relationships among genotypes.

In addition, the Comprehensive Selection Scheme using 'multi-trait selection master' of the GGE biplot (Yan and Fregéau-Reid, 2008) was employed to select tolerant inbreds for hybrid production and population improvement under both research conditions. Specifications were made for single trait selection rate of 2%, independent culling rate of 30%, and overall culling rate of 80% of the entries using the six selected traits (YD, EPP, ASI, PA, EA, and LS). Weights of +1 and +0.5 were assigned to YD and EPP, respectively; while -0.5 was assigned to the remaining four traits for data on drought studies. The same was done for data on low-N studies except that a weight of -0.25 was assigned to each of LS1 and LS2. The GGE bipo-

lot model equation is represented below:

$$\frac{\hat{Y}_{ij} - \mu - \beta_j}{d_j} = \lambda_1 g_{i1} e_{1j} + \lambda_2 g_{i2} e_{2j} + e_{ij}$$

where: $\hat{Y}_{ij}$ is the genetic value of the combination between inbred i and trait j; $\mu$ is the mean of all combinations involving trait j; $\beta_j$ is the main effect of trait j; $\lambda_1$ and $\lambda_2$ are the singular values for PC1 and PC2; $g_{i1}$ and $g_{i2}$ are the PC1 and PC2 eigenvectors, respectively, for inbred i; $e_{1j}$ and $e_{2j}$ are the PC1 and PC2 eigenvectors, respectively, for trait j; $d_j$ is the phenotypic standard deviation; and $e_{ij}$ is the residual of the model associated with the combination of inbred i and trait j.

The grain yield data for the single cross hybrids were subjected to genotype main effect plus genotype × environment interaction (GGE) biplot analysis to decompose the G x E interactions of the single-cross hybrid experiment (Yan et al, 2000; Yan, 2001). The GGE biplot was used to obtain information on the hybrids that were suitable across stress and non-
stress environments and to investigate the stability of hybrids in the drought stress and well-watered environments. These analyses were done using GGE biplot software, a Windows application that fully automates biplot analysis (Yan, 2001). The program is available at www.ggebiplot.com (verified 24 February 2011). The GGE biplot model 2 equation used is as follows:

\[ \hat{Y}_i - \bar{Y} = \lambda_1 x_{i1} + \lambda_2 x_{i2} + \epsilon_i \]

In this model, \( \hat{Y}_i \) is the average yield of genotype \( i \) in environment \( j \); \( Y \) is the average yield across all genotypes in environment \( j \); \( \lambda_1 \) and \( \lambda_2 \) are the singular values for PC1 and PC2; \( x_{i1} \) and \( x_{i2} \) are the PC1 and PC2 scores, for genotype \( i \); \( \eta_{1j} \) and \( \eta_{2j} \) are the PC1 and PC2 scores, for environment \( j \); \( \epsilon_i \) is the residual of the model associated with the genotype \( i \) in environment \( j \).

**Results**

**Analysis of variance**

Results of the analysis of variance of inbreds across years and locations under drought stress showed significant genotype (G), environment (E), and G x E interaction (GEI) mean squares for most traits (See supplementary Table 1). Genotype mean squares for HC and RL and GEI mean squares for EA, HC, and RL for GEI were not significant (See supplementary Table 1). Similarly mean squares of inbreds were significant for all traits under low-N conditions except ASI, SL, and EPP for genotypes, and DS, DA, and SL for environments. In contrast, GEI mean squares of inbreds were significant for only YD, EH, PA, HC, and leaf senescence at 8 WAP (LS1) and 10 WAP (LS2) (See supplementary Table 1). Mean YD of the inbreds ranged from 646 kg ha\(^{-1}\) for TZEEI42 to 1,823 kg ha\(^{-1}\) for TZEEI6 under drought stress whereas, it ranged between 890 kg ha\(^{-1}\) for TZEEI91 and 3,281 kg ha\(^{-1}\) for TZEEI36 under well-watered conditions. Under low N, the range was 153 kg ha\(^{-1}\) for TZEEI4 for 2,428 kg ha\(^{-1}\) for TZEEI45 while under high-N conditions, it ranged between 719 for TZEEI5 and 2550 for TZEEI45 (See supplementary Table 1).

The ANOVA combined across stress and non-stress environments for the hybrids revealed significant G, E, and GEI mean squares for most traits (See supplementary Table 1). The top ranking hybrid TZEEI29 x TZEEI21 out-yielded the best open-pollinated check, 2000 Syn EE-W STR by 15% under drought stress and 6% under well-watered conditions. On average, drought stress reduced YD by 59% (See supplementary Table 2), indicating that a severe level of moisture stress was achieved in this study.

**Table 2**

<table>
<thead>
<tr>
<th>DA</th>
<th>PH</th>
<th>EH</th>
<th>RA</th>
<th>EA</th>
<th>ASI</th>
<th>EPP</th>
<th>LS</th>
<th>YD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9±0.05**</td>
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* ** Significant at 0.05 and 0.01 level of probabilities, respectively.
† Genetic correlations could not be estimated * because variance was less than or equal to zero.

The presence of significant genotypic mean squares for grain yield and all the drought and low-N adaptive traits (EPP, ASI, EA, PA, and stay green characteristics) of the inbreds justified the use of the Comprehensive Selection Scheme (Yan and Fregeau-Reid, 2008) and the GT biplot to identify the best candidate parents for hybrid production and for the selection of the inbreds with favorable alleles for introgression into breeding populations. Similarly, the highly significant GEI justified the use of the GGE biplot to identify the most stable and high yielding hybrids, using the codes in the figures and tables in the following sections.

**Selection of drought and low-N tolerant inbreds**

In an effort to identify inbreds with superior individual traits that could serve as sources of desirable alleles for improving breeding populations for drought stress, three approaches were used, including trait profile analysis, a comprehensive selection scheme using multi-trait selection master, and mean vs stability view of the GT biplot.

**Trait profile analysis**

The polygon view of the GT biplot was used for trait profile analysis. In the GT biplot displays (See supplementary Figure 1), Principal Component (PC) 1 and PC2 explained 45.6% of the total variation among the traits of the inbreds under drought stress. In the polygon view in Figure 1, the vertex inbred in each sector represents the inbred with the highest values for the traits that fall within that particular sector. The distance between the genotype and the biplot origin (centre-point of the biplot), is a measure of the geno-
type’s peculiarity (i.e., how it differs from the mean of all genotypes), which is a hypothetical genotype that has an average level for all traits and is represented by the biplot origin. Therefore, genotypes at the vertex of a sector have extreme levels for one or more traits and even though they may or may not be a superior variety, they may be useful as sources of genes for those traits (Yan and Fregeau-Reid, 2008).

The transformed view of supplementary Figure 1 displayed the cultivars that had the lowest values for one or more traits (Figure not presented). Hence, traits that contribute to the performance of an inbred can be graphically identified by both biplots. Thus, TZEEI 6 was the vertex inbred for YD but had undesirable PH while TZEEI39 was the vertex inbred for EPP and was therefore superior in terms of this trait. The other vertex inbreds, TZEEI23, TZEEI69, TZEEI91, TZEEI42, and TZEEI88, had high values for the traits that fell in their respective sectors and were therefore poorest in terms of the traits (Figure 1). In addition to the superior YD, TZEEI6 had also the lowest values for EA and PA and was therefore superior in these traits. Similarly, TZEEI39 had the lowest values for DS, DA, ASI, and LS, thus indicating its superiority in these traits, in addition to the high EPP. Even though TZEEI88 had undesirable EH (See supplementary Figure 1), it had the lowest value for HC and could be a good source inbred for improvement of the trait in breeding programs (Figure not presented).

Under low-N conditions, TZEEI45 was the best inbred in terms of YD and EPP, while the vertex inbreds, TZEEI19, TZEEI51, TZEEI23, TZEEI76, TZEEI73, and TZEEI27, had high values for traits in their sectors implying that they were inferior in these traits (Figure 2). In addition to the high YD and EPP of TZEEI45 in Figure 2, the reversed view (Figure not presented) showed that it had the lowest values for EA, PA, HC, ASI, and LS2. Furthermore, TZEEI9 was the vertex inbred for DA and DS, while TZEEI83 was the vertex inbred for SL, indicating that they were superior in these traits and could be used as sources for improving other extra-early inbreds and populations for these traits (See supplementary Figure 2).

**Comprehensive Selection Scheme using Multitrait Selection Master**

Under drought conditions, 22 of the 90 extra-early

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**Figure 3** - A “which-won-where or which-is-best-at-what” based on a genotype x environment yield data of 36 extra-early maturing maize hybrids evaluated in two environments in 2009. The biplot was based on genotype-focused singular value partitioning (“SVP = 2”) and is therefore appropriate for visualizing the relationships among environments. Principal component PC1 and PC2 explained 100% of yield variation. DD: drought stress, WW: well-watered conditions. Hybrids’ code: 1 = TZEEI29/TZEEI21; 2 = TZEEI4/TZEEI21; 3 = TZEEI29/TZEEI14; 4 = TZEEI29/TZEEI37; 5 = TZEEI39/TZEEI90; 6 = TZEEI95/TZEEI13; 7 = TZEEI67/TZEEI37; 8 = TZEEI29/TZEEI54; 9 = TZEEI29/TZEEI67; 10 = TZEEI4/TZEEI14; 11 = TZEEI4/TZEEI37; 12 = TZEEI6/TZEEI14; 13 = TZEEI95/TZEEI90; 14 = TZEEI39/TZEEI14; 15 = TZEEI67/TZEEI14; 16 = TZEEI95/TZEEI49; 17 = TZEEI4/TZEEI49; 18 = TZEEI4/TZEEI49; 19 = TZEEI39/TZEEI37; 20 = TZEEI95/TZEEI76; 21 = TZEEI95/TZEEI63; 22 = TZEEI95/TZEEI58; 23 = TZEEI79/TZEEI76; 24 = TZEEI62/TZEEI95; 25 = TZEEI79/TZEEI85; 26 = TZEEI62/TZEEI79; 27 = TZEEI82/TZEEI76; 28 = TZEEI82/TZEEI85; 29 = TZEEI95/TZEEI78; 30 = TZEEI95/TZEEI79; 31 = TZEEI79/TZEEI63; 32 = TZEEI82/TZEEI95; 33 = TZEE-W Pop STR C4; 34 = TZEE-Y Pop STR C4; 35 = 2000 Syn EE-W; 36 =2004 TZEE-W STR C4.
In this study, TZEE16 was the closest to the ideal and was thus the best inbred. The inbreds TZEE14, TZEE36, and TZEE38, were also located in the innermost concentric circle and were identified as ideal inbreds (Figure 1) under drought stress. Under drought stress, TZEE16 was the best performing inbred based on multiple traits, whereas TZEE42 was the worst (Figure 1). The inbreds TZEE42, TZEE161, TZEE150, TZEE97, TZEE98, TZEE91, TZEE167, TZEE178, and TZEE148, were ranked as the lowest in performance and could serve as susceptible inbreds as well as parents for drought susceptible hybrid checks for drought trials (Figure 3).

Under low-N conditions, TZEE19 was ranked closest to the ideal inbred, based on the selected traits (Figure 4). The inbreds TZEE19 and TZEE56, were also located around the innermost concentric circle and could be considered as top ranking under low-N conditions. The inbreds TZEE14, TZEE29, TZEE46, and TZEE49, were identified as tolerant to both drought stress and low N by the Comprehensive Selection Scheme.

In the GT biplot displays (Figures 1 and 2), the thick single-arrow black line that passes through the biplot origin (intercept of the vertical and horizontal axis) and the average tester (centre of the innermost concentric circle with an arrow) is referred to as the average-tester axis (ATC). The double-arrow line (ATC ordinate) separates entries with below-average means (to the left side of the line) from those with above-average means. The average performance of an inbred is approximated by the projection of its marker on the ATC. The ideal inbred is described as the entry with longest projection onto ATC abscissa and positioned closest to the ideal entry (innermost concentric circle with an arrow). Based on the multiple traits assayed in this study, TZEE16 was the closest to the ideal and was thus the best inbred. The inbreds TZEE14, TZEE36, and TZEE38, were also located in the innermost concentric circle and were identified as ideal inbreds (Figure 1) under drought stress. Under drought stress, TZEE16 was the best performing inbred based on multiple traits, whereas TZEE42 was the worst (Figure 1). The inbreds TZEE42, TZEE161, TZEE150, TZEE97, TZEE98, TZEE91, TZEE167, TZEE178, and TZEE148, were ranked as the lowest in performance and could serve as susceptible inbreds as well as parents for drought susceptible hybrid checks for drought trials (Figure 3).

Under low-N conditions, TZEE19 was ranked closest to the ideal inbred, based on the selected traits (Figure 4). The inbreds TZEE19 and TZEE56, were also located around the innermost circle and could be considered as top ranking under low-N conditions. The inbreds TZEE14, TZEE29, TZEE46, and TZEE49, were identified as tolerant to both drought stress and low N by the Comprehensive Selection Scheme.

low-N susceptible hybrid checks for use in trials (Figure 4).

**Hybrid performance**

The GGE biplot polygon view in Figure 3 revealed that a hybrid (Hyb 1) was the best genotype under drought stress whereas an open-pollinated cultivar (coded 36) was the best under well-watered conditions. Presented in Figure 4 is the “mean-vs-stability” view of the GGE biplot. In this figure, the axis of the AEC abscissa (average environment axis) is the single-arrowed line that passes through the biplot origin and the “average environment axis” which is at the center of the small circle. The axis of the AEC ordinate is the double-arrowed line through the biplot origin and is perpendicular to the AEC abscissa. The arrow shown on the axis of the AEC abscissa points in the direction of higher mean performance of the genotypes and as a result ranks the genotypes with respect to the mean performance. Based on this, 18 genotypes produced above-average grain yield and may be ranked as follows: Hyb36 > Hyb23 = Hyb33 = Hyb11 = Hyb7 = Hyb12 < Hyb35 = Hyb9 = Hyb24 = Hyb26 = Hyb10 = Hyb3 = Hyb14 = Hyb4 = Hyb5 = Hyb2 = Hyb25 = Hyb21. Of the 18 genotypes, hybrids 1, 12, 24, 26, and 34 were identified as very stable while the highest yielding open-pollinated cultivars, 2004 TZEE-W Pop STR C4, and TZEE-W Pop STR C4 and the hybrid TZEEI79 x TZEEI76 were the least stable. In maize production, an ideal genotype should have both high mean performance and high stability within a mega-environment. Thus, TZEEI29 x TZEEI21 was the closest to the ideal genotype and may be considered as the best drought tolerant hybrid.

**Genotypic and phenotypic correlation analyses**

**Inbreds**

Grain yield had significant genotypic correlation coefficients with all measured traits under drought stress except RL, SL, HC, and LS. In contrast, genotypic correlation coefficients between grain yield and other traits were not significant under drought stress (Table 2). Only eight of the 78 possible genetic correlation coefficients were statistically significant and these involved DA, DS, PH, EH, ASI, and EA interaction. In general, the coefficients were larger than the phenotypic correlations.

Under low-N environments, significant phenotypic correlations existed between grain yield and all other traits except ASI and SL (See supplementary Table 3). Similarly, strong genetic correlations were obtained between grain yield and all other traits except DA and DS. The genetic correlation coefficients between grain yield and several other traits under low-N were large, especially those involving ASI, HC, PA, LS1, LS2, EA, and EPP. Here also, the genetic correlation coefficients were generally larger than the phenotypic correlations, and only seven of the 78 possible coefficients were significant statistically.

**Hybrids**

Grain yield of hybrids had significant genotypic and phenotypic correlation coefficients with PH, EH, and EPP in both drought stress and well-watered conditions (See supplementary Tables 4 and 5). The traits, PA and EA showed significant genetic correlation with yield under drought whereas DS and ASI were genetically correlated with grain yield under well-watered conditions. Phenotypic correlation was significant between grain yield and DS, HC, and EA under drought stress, the correlation was significant for ASI under well-watered conditions.

**Inbred-Hybrid correlation and regression analyses**

Grain yield of inbred lines had significant correlation (r= 0.29) with F1 hybrid yield performance under drought stress but not under well-watered environments (See supplementary Table 6). When evaluated under drought stress, mid-parental values for grain yield resulted in only modest prediction of hybrid grain yield; that is, 1 kg ha\(^{-1}\) increase in mid-parent grain yield resulted in only 0.317 kg ha\(^{-1}\) increase in hybrid grain yield under drought stress (Figure not presented). Mid-parent values for DS and ASI under drought stress had significant correlation with F1 hybrid yield under well-watered environments (See supplementary Table 6). Each additional day increase in mid-parent values of DS and ASI under drought stress reduced hybrid grain yield in well-watered environments by 182 and 485 kg ha\(^{-1}\), respectively (See supplementary Figure 3). Inbred performance under well-watered environments had no significant correlation with hybrid yield performance either under well-watered or drought stress environments, but hybrid yield under drought had significant positive correlation with hybrid yield under well-watered conditions. Regression models showed that high-yielding hybrids under drought stress will be high-yielding under well-watered environments but to a limited extent if vice versa (Figures not presented).

**Discussion**

To improve maize productivity and food security in the savannas of WCA, there is an urgent need for extra-early maturing open-pollinated maize varieties and hybrids that are not only capable of escaping drought but can also tolerate mid-season drought that occurs during the flowering and grain-filling periods as well as tolerate low-N. Genotypic correlations between traits are of interest to breeders selecting for improved grain yield of extra-early maize under drought stress or low-N because they indicate the direction and magnitude of correlated responses to selection, the relative efficiency of indirect selection and allow the computation of appropriate multiple trait selection indices (Falconer and Mackay, 1996). The large and significant genetic and phenotypic correlations between grain yield and most traits under low-N environments suggest that there may be considerable genetic association between grain yield and...
most of the characters studied. Similarly, the significant phenotypic correlations between grain yield and most traits as well as the significant genetic correlations among some stress-adaptive secondary traits, including ASI, under drought stress suggest considerable genetic association among the traits. The weak genetic correlations between grain yield and other traits under drought stress in this study could be due to the poor performance of the extra-early inbreds under severe drought stress (mean grain yield of 1819 kg ha$^{-1}$). In this case, the environment might have masked the genetic variances and covariances between grain yield and each of the other traits as indicated from earlier studies by Badu-Apraku et al (2004, 2005). This hypothesis seems to be supported by the fact that most of the phenotypic correlation coefficients involving grain yield and other traits under drought stress were significant. The lack of significant phenotypic and genotypic correlations between grain yield and stay green characteristic observed in this study is consistent with the results of Badu-Apraku et al (2011). Similarly, the strong genetic correlation observed between grain yield and most measured traits under low-N is in agreement with the findings of previous studies. For example, strong correlations have been reported for traits such as maturity, plant height, and 100-kernel weight under low-N stress (Lafitte and Edmeades, 1995). Also, Badu-Apraku et al (2011) in a study of the interrelationship of grain yield and other traits of early maturing maize cultivars under low-N, reported strong correlation between grain yield, days to silk, days to anthesis, stay green characteristics, ASI, plant height, ears per plant, ear aspect and plant aspect. The significant genotypic mean squares detected for YD and most other traits for the inbreds under both managed drought stress and low-N conditions indicated wide genetic variability among the inbreds for the measured traits under both stress environments. This result was supported by the wide range in YD (854 kg ha$^{-1}$ for TZEEI42 to 1819 kg ha$^{-1}$ for TZEEI6) under drought stress and (200 kg ha$^{-1}$ for TZEEI4 to 2,181 kg ha$^{-1}$ for TZEEI45) under low-N conditions, thus suggesting wide genetic variability for tolerance to both drought stress and low-N in the inbreds. Similarly, the wide ranges observed for measured traits of the extra-early inbreds under drought stress and low-N is in support of our finding that wide genetic variability for grain yield and stress adaptive traits exist among the extra-early inbreds under both stress environments. This finding is further reinforced by the linear regression between the inbred line per se yield and hybrid yield, particularly under induced drought stress. The significant genotypic differences among the inbreds in the measured traits were expected as the inbreds were derived from broad based germplasm sources. The significant genotypic mean squares for YD and all the drought and low-N adaptive traits (EPP, ASI, EA, PA, and LS1 and LS2) suggested that genes for tolerance to low-N and drought stress at flowering and grain-filling periods are present in the extra-early inbreds evaluated in this study, and that it should be possible to select for both drought and low-N tolerant extra-early maize among the inbreds. This prompted the use of the GT biplot to identify the superior inbred lines under both research conditions. The identification of the 22 out of 90 extra-early inbreds with superior performance under managed drought stress at the flowering and grain-filling periods in this study has provided evidence in support of the hypothesis that, after several years of cultivation in the heat and drought stress environments of the Sudan savanna of WCA, selected extra-early inbred lines of the IITA maize program have adaptive traits for tolerance to drought stress. They are not only capable of escaping drought stress but also possess drought tolerant genes that will allow them to withstand the mid-season drought that occurs during the flowering and grain-filling periods in the savannas of WCA. The hypothesis was further confirmed by the superior performance of the 22 selected drought tolerant inbreds in hybrid combinations. It is striking to note that 15 of the extra-early inbreds were identified as tolerant to low-N even though no conscious effort was ever made to select the lines for this trait. This result is in agreement with the findings of Bänziger et al (1999) who showed that improvement for drought tolerance also resulted in specific adaptation and improved performance under low-N conditions, suggesting that tolerance to either stress involves a common adaptive mechanism. Goodman (1985) reported that the choice of breeding materials determines the future success of a breeding program. Therefore, the extra-early inbreds identified under moisture stress and/or low-N conditions in this study, may be used directly as parents in our hybridization program to generate stable hybrids for the farmer, or crossed with other adapted germplasm to increase the frequency of favorable alleles for drought tolerance (Beck et al, 1997; Menkir and Akintunde, 2001) and low-N tolerance.

The reduction in grain yield of the extra-early hybrids under drought stress ranged from 48% for TZEEI95 x TZEEI78 and TZEEI82 x TZEEI58 to 70% for TZEEI82 x TZEEI76 with an average reduction of 59% for all hybrids and open-pollinated checks. Badu-Apraku et al (2010) reported only 44 % reduction in grain yield of early maturing cultivars evaluated under drought stress at Ikenne and Bagauda from 2007 to 2009 and attributed this to the moderate level of drought stress achieved at Bagauda in 2008 due to an unusually high rainfall during crop growth. It is striking to note that the level of drought stress in this study was comparable (39-90%, with an average of 61%) to that found in the early maturing hybrid trials conducted under similar conditions at Ikenne in our breeding program; a further confirmation of the presence of drought tolerant alleles in the extra-early inbreds.
In an effort to explore the possibility of using information on inbreds to predict the potential hybrid performance, under drought stress and thereby reduce the need for extensive hybrid evaluation, correlation between hybrid grain yield and mid-parent values of grain yield and other traits as well as regression analyses were performed. The highly significant positive correlation of the inbred per se yield performance with F1 hybrid yield performance under drought stress suggests the presence of additive gene action in the inbreds (Betran et al., 2003; Gethi and Smith, 2004). The implication is that the inbred lines should transmit their yield potentials to their hybrids. However, caution should be exercised in the interpretation of this data since the R-square value from the regression analysis is relatively small. Despite the positive correlations between the inbreds and hybrids in drought stress environments, comparative yield performance of the hybrids will still be necessary, especially because of the fact that inbred performance under well-watered environments had non-significant correlation with hybrid yield performance under well-watered or drought stress environments. Results of regression analysis revealed that hybrid yield under drought stress adequately predicted the performance of the hybrid under well-watered environments.

In conclusion, for the first time, low-N tolerant extra-early inbreds and drought-tolerant extra-early inbreds and hybrids are now available in sub-Saharan Africa. These inbreds are not only drought escaping (a characteristic of extra earliness), but carry genes that are tolerant to drought at flowering and grain-filling periods. Lastly, inbreds and hybrids combining tolerance to both stresses are also now available. The availability of the low-N and drought tolerant extra-early inbreds and hybrids should go a long way in reducing the instability of maize yields in sub-Saharan Africa especially in the savannas as well as during the second season in the forest ecologies.

Acknowledgements

Financial support of the Drought Tolerant Maize for Africa (DTMA) Project for this study is gratefully acknowledged. The authors are also grateful to Mrs R Umelo for editorial assistance. Special thanks are due to the staff of the IITA Maize Program in Ibadan, Nigeria, for technical assistance.

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