

Article

Genetic Diversity, Population Structure and Inter-Trait Relationships of Combined Heat and Drought Tolerant Early-Maturing Maize Inbred Lines from West and Central Africa

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Abstract: Adequate knowledge and understanding of the genetic diversity and inter-trait relationships among elite maize inbred lines are crucial for determining breeding strategies and predicting hybrid performance. The objectives of this study were to investigate the genetic diversity of 162 early maturing white and yellow tropical maize inbred lines, and to determine the population structure, heterotic groups and inter-trait relationships among the lines. Using 9684 DArT single nucleotide polymorphism (SNP) markers, a gene diversity (GD) of 0.30 was recorded for the inbred lines with polymorphic information content (PIC) ranging from 0.08 to 0.38. The genetic relatedness among the inbred lines evaluated revealed six different groups based on the history of selection, colour of endosperm and pedigree. The genotype-by-trait (GT) biplot analysis identified inbred 1 (TZEI 935) as outstanding in terms of combined heat and drought (HD) tolerance with the base index analysis identifying 15 superior inbreds in the HD environment. A wide range of genetic variability was observed among the inbred lines, indicating that they are an invaluable resource for breeding for HD tolerance in maize breeding programmes, especially in West and Central Africa.

Keywords: genetic diversity; DArT SNP markers; maize; early maturity; heat and drought tolerance

1. Introduction

Molecular analyses in combination with morphological and agronomic evaluation of germplasm are useful for providing complementary information on the genetic diversity in a breeding programme [1]. Thus, monitoring of genetic diversity in the base material helps breeders to formulate breeding strategies by selecting parental combinations for advance selection [2], determining the level of genetic variability whilst identifying the main clusters selected for specific traits [3]. Information on genetic diversity is also useful in classifying inbred lines into heterotic groups [4,5], and predicting hybrid performance [6]. By definition, a heterotic group encompasses a set of directly related inbred lines which produce high yielding and vigorous hybrids when crossed to inbred lines from different heterotic groups, but not when crossed to inbred lines from the same heterotic group [7].

Additionally, the availability of information on the genetic diversity and population structure facilitates the assessment of the likelihood of loss in genetic diversity during preservation or selection [8],

as well as assessment of the relative strengths of evolutionary forces [9,10]. Molecular marker technology has been a resourceful technique for assessing genetic diversity and delimiting heterotic groups [11].

Previous studies have reported a strong association between molecular marker-based genetic distance and hybrid performance in maize [11,12]. However, these findings should be used with caution as it is not always that these findings can predict hybrid performance, heterosis and specific combining ability (SCA) of single cross hybrids [13,14]. Recent studies involving early (90–95 days to physiological maturity) and extra early (80–85 days to physiological maturity) maize inbred lines have convincingly shown that genetic variability is of utmost significance in demonstrating heterosis [15,16]. Primarily, inbred lines are developed by crossing the best lines from the same heterotic group followed by inbreeding and selection, whereas hybrids are developed by crossing lines from dissimilar heterotic groups [17]. When lines lose vigour over time as a result of inbreeding depression, there is the need to bulk-sib within them to regain an acceptable level of vigour to ensure survival. The concept of heterosis is very important in the development of climate-smart maize inbred lines and hybrids [18]. Climate-smart inbred lines are able to tolerate the major stresses (for example, drought, heat and low soil fertility) that limit their growth. Identification of stress-tolerant lines and their utilization in the development of productive hybrids has been found to be a sustainable means of developing climate-resilient maize varieties.

During the past decade, molecular markers, especially simple sequence repeat (SSR) markers, have been widely used to study the correlations among hybrid performance and genetic distance [15,19]. Recently, Richard et al. [20] successfully used single nucleotide polymorphism (SNP) markers to classify 45 maize inbred lines of temperate and tropical origin into four heterotic groups corresponding to the Salisbury white (N), Southern cross (SC), Iowa stalk synthetic (BSS) and the Lancaster heterotic groups. Based on the results, it was possible to demonstrate that the genetic divergence of the temperate inbreds was greater than that of the tropical inbreds. Similarly, the SNP marker method used for grouping inbred lines into heterotic groups has been shown to be more efficient than the SCA and general combining ability (GCA) methods, as well as the heterotic grouping based on GCA of multiple traits (HGCAMT) [21].

During the past two decades, the International Institute of Tropical Agriculture (IITA) early and extra-early maturing maize breeding programme (IITA-MBP) has focused on developing germplasm with improved resistance/tolerance to multiple stresses, including drought, and combined HD stress tolerance [22]. This strategy has resulted in the development of broad-based normal endosperm maize populations including TZE-W Pop DT STR and TZE-Y Pop DT STR in the early maturity group and TZEE-Y Pop DT STR and TZEE-W Pop DT STR within the extra-early maturity group [23,24]. The National Agricultural Research Systems (NARS) and farmers in different agro-ecological zones of West and Central Africa (WCA) have widely adopted outstanding maize hybrids and synthetic varieties developed from inbred lines with desirable agronomic characteristics that have adapted to multiple-stress environments [15]. The current resurgence of climate variability especially in WCA has made the development of drought and combined HD-tolerant maize varieties in the sub-region very critical [22]. It is anticipated that this would lead to accelerated achievement of food security and improved livelihood of resource poor farmers in WCA.

Another important focus of this study was to understand which traits have the maximum likelihood of complementing grain yield of the inbred lines. This information was necessary because high grain yield of inbred *per se* [25], coupled with other desirable agronomic characteristics, are major criteria for selecting an inbred line as a suitable seed parent. This trait could be transferred to their hybrids and such information could facilitate prediction of superior hybrid performance, including excellent plant and ear aspects which are highly heritable, and delayed stay-green characteristic under combined heat and drought stress. Therefore, analysis of inter-relationships among traits of maize genotypes is crucial to maize improvement programmes involved in genetic enhancement of grain yield under combined HD stress. This is because most economic traits of interest in maize including grain yield have low heritability because the inheritance is quantitative. In view of this, gains from direct selection for such

traits are very low. However, this problem could be mitigated through indirect selection of secondary traits that have high genetic correlations with grain yield coupled with high heritability and that are easily measured. Yan and Kang [26] proposed genotype-by-trait (GT) analysis for multiple trait-based assessment of genotypes. Such statistical tools aid in the identification of genotypes with specific desirable traits that could be employed as a genetic resource in a breeding programme or released for commercialization while also presenting a graphical display of the genetic correlations among traits [27,28]. Thus, the objectives of this study were (1) to assess the genetic diversity and population structure of selected heat- and drought-tolerant early maturing white and yellow maize inbreds using DArT SNP markers and (2) examine the interrelationships among traits of the early maturing inbreds in an effort to identify those strongly associated with grain yield in maize under combined HD stress.

2. Materials and Methods

2.1. Description of Germplasm

A total of 162 early maturing maize inbred lines developed in the IITA-MBP, Ibadan, Nigeria, with tolerance to drought and combined heat and drought stress were used in the present study (Table 1). The IITA inbred lines were extracted from the broad-based populations (TZE Comp5-Y C₆, TZE-W P_{OP} STR 104 C₀, TZE-W P_{OP} STR 107 C₀, TZE-Y P_{OP} STR 106 C₀, TZE-W P_{OP} STR 108 C₀, TZE-W Pop STR C₀, TZE-W Pop × LD C₀ and TZE-Y Pop STR C₀) developed from exotic and local germplasm identified as a result of many years of extensive testing for broad adaptation to dry and hot climates of the savannas of WCA. Furthermore, some of the inbred lines were derived from crosses between the broad-based drought-tolerant population, TZE COMP 3 DT C₂ F₂, and elite *Striga* resistant inbred lines, TZEI 18, TZEI 4, TZEI 60 and TZEI 98, as well as crosses between the broad-based *Striga* resistant but drought-susceptible population, TZE COMP 5-W DT C₇, and the drought tolerant inbred lines TZEI 2, TZEI 18, TZEI 31, TZEI 56 and TZEI 65. In addition to the 34 lines derived from two IITA bi-parental populations [(TZEI 11 × TZEI 8) and (TZEI 7 × TZEI 3)], the genetic materials for this study also included three inbred lines derived from two CIMMYT broad-based populations (M37W/ZM607 and Cuba/Guad C₃).

Table 1. Source populations of 162 maize inbred lines used in the genetic diversity study.

S/N	Source of Population	Number of Extracted Inbred Lines	Grain Colour
1	(TZE COMP 3 DT C ₂ F ₂ × TZEI 18) S8	1	White
2	(TZE Comp 3 DT C ₂ F ₂ × TZEI 4) S8	4	White
3	(TZE COMP 3 DT C ₂ F ₂ × TZEI 60) S8	5	White
4	(TZE COMP 3 DT C ₂ F ₂ × TZEI 98) S8	12	White
5	(TZE COMP 5-W DT C ₇ × TZEI 18) S8	1	White
6	(TZE COMP 5-W DT C ₇ × TZEI 2) S8	10	White
7	(TZE COMP 5-W DT C ₇ × TZEI 31) S8	4	White
8	(TZE COMP 5-W DT C ₇ × TZEI 56) S8	5	White
9	(TZE COMP 5-W DT C ₇ × TZEI 65) S8	17	White
10	(TZEI 11 × TZEI 8) S8	27	Yellow
11	(TZEI 7 × TZEI 3) S8	7	White
12	[M37W/ZM607#bF37sr-2-3sr-6-2-X]-8-2-X-1-BB-B-xP84c1	1	Yellow
	F27-4-3-3-B-1-B] F29-1-2-2 × [KILIMA		
	ST94A]-30/MSV-03-101-08-B-B-1xP84c1 F27-4-1-4-B-3-B]		
13	F2-1-2-1-1-1-B × CML486]-1-1	1	White
	[M37W/ZM607#bF37sr-6-2-X]-8-2-X-1-BB-B-xP84c1		
	F27-4-3-3-B-1-B] F29-1-2-1-6 × [KILIMA		
14	ST94A]-30/MSV-03-2-10-B-1-B-B-xP84c1	1	White
	F27-4-1-6-B-5-B]3-1-2-B/CML442)-1-1		
	Cuba/Guad C ₃ F85-3-3-1-B-B-B-B		
15	TZE Comp5-Y C ₆ S8	2	Yellow
16	TZE-W POP STR 104 S8	2	White
17	TZE-W POP STR 107 S8	12	White
18	TZEE-Y POP STR 106 S8	3	Yellow
19	TZE-Y POP STR 106 S8	4	Yellow
20	TZE-W POP STR 104 S8	11	White
21	TZE-W POP STR 108 S8	15	White
22	TZE-W Pop STR Co S8	3	White
23	TZE-W Pop × LD S8	1	White
24	TZE-Y POP STR 106 S8	7	White
25	TZE-Y Pop STR Co S8	5	Yellow
26	WEC STR S8 Inbred	1	White

2.2. DNA Extraction and Genotyping

The 162 early maturing inbred lines were planted at the IITA research field, Ibadan, Nigeria under optimal growing conditions in 2018. At two weeks after planting, leaf samples were collected from each of the inbred lines, dried in a Labconco Freezone 2.5 L System lyophilizer (Marshall Scientific, Hampton, NH, USA) and ground using a Spex™ Sample Prep 2010 Geno/Grinder (Thomas Scientific, Swedesboro, NJ, USA).

2.3. Data Filtering and Analysis

Genomic DNA samples were extracted from freeze-dried leaf tissues of each inbred line using the DArT protocol (www.diversityarrays.com/files/DArTDNAisolation.pdf) as previously reported [18]. The quality of the DNA sample was determined using a 1% agarose gel test whilst the quantity was measured using the Nanodrop system (Thermo Scientific, Swedesboro, NJ, USA). Genotyping was performed at the IGSS-BecA-ILRI Platform, Nairobi, Kenya.

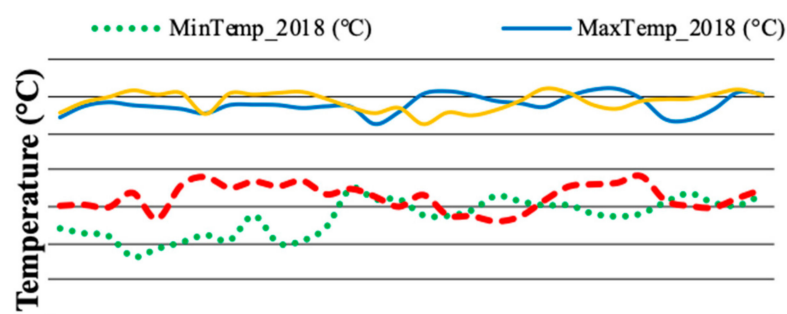
Following the methodology previously described [15,29], the DArT-SNP markers with missing data above 5% and 20% heterozygosity and minor allele frequency of below 0.05 were removed. From the initial DArTseq markers obtained from sequencing, a total of 9684 SNP markers distributed across the 10 maize chromosomes were retained for all subsequent analyses. The major allele frequency, polymorphic information content (PIC), number of alleles, heterozygosity and the gene diversity were calculated using Power Marker software V3.2.5 [30]. Analysis of the population structure was done by employing the admixture model using the STRUCTURE 2.3.4 software package [31]. The results from the model were achieved by changing the number of clusters (K) from one to twenty with ten to twenty adjustments for each K and subjecting the data to 10,000 Markov Chain Monte Carlo and 10,000 burn-in as described previously [15,25,29]. The most appropriate K value was identified by running the data obtained into the STRUCTURE HARVESTER using the Evanno method [32,33]. Each inbred was allocated to a group using a cut off threshold of 70%, while inbreds showing less than the designated threshold value were assigned to an additional group described as the mixed group.

Subsequent to verification of the number of sub-populations using the STRUCTURE, the phylogenetic relationship among the inbred lines was accessed using the neighbor-joining (NJ) method under 30,000 bootstraps in the DARwin software [34]. The genetic distance matrix was obtained using the Jaccard similarity test [35] using the formula $d_{ij} = (a + b) / (a + (a + c))$, where d_{ij} is the dissimilarity between units i and j , a is the number of variables where X_i and X_j are present, b is the number of variables where X_i is present and X_j is absent, c is the number of variables where X_i is absent and X_j is present.

2.4. Assessment of the Performance of the Inbreds Under Combined Heat and Drought Stress Conditions

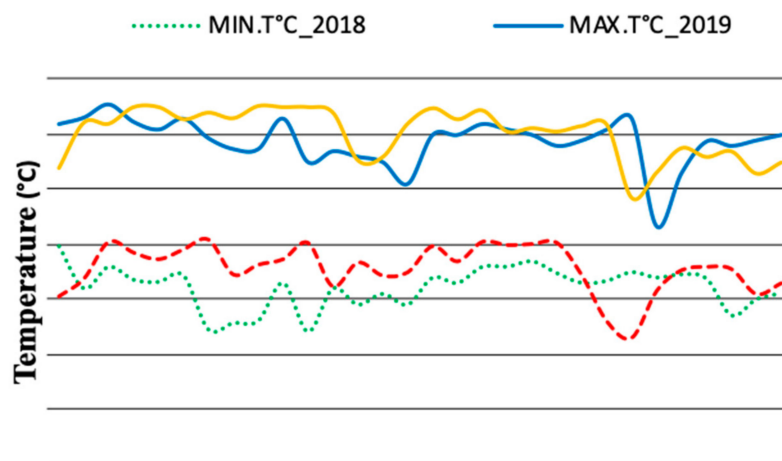
The set of 162 inbred lines were planted at Manga (11° 3'N, 0° 14'W, altitude 252 m, 800 mm and 1,100 mm annual rainfall) in the Upper East Region of Ghana and Kadawa (11°45' N, 8°45' E, altitude 468.5 m, 884 mm annual rainfall) in the Kano state of Nigeria during the dry seasons (February to May) of 2018 and 2019. The maize plants were exposed to intense DH stress for 2-3 weeks in April at Kadawa and Manga, with the day temperatures of 36 to 41.2 °C and night temperatures of 18 to 27.4 °C for Kadawa (Figure 1), while at Manga the day and night temperatures were 31.6 to 42.8 °C and 22.0 to 29 °C, respectively (Figure 2). A 9 × 18 alpha lattice design with two replications was used for the inbred experiments. Single row plots each 4 m long, with a spacing of 0.75 m between rows and 0.40 m within rows, constituted an experimental unit. Three seeds were planted per hill and later thinned to two plants per stand to obtain a population density of about 66 666 per ha. No rainfall was recorded at Kadawa during the grain filling period of April 2018 and 2019, resulting in the highest day temperature throughout the growth cycle of the plants (Table 2). Similarly, at Manga, although some amount of rainfall was recorded in the month of April during 2018 and 2019, the highest day and night temperatures recorded during the growth cycle of the plants were obtained in April

(Table 2). The maize plants were irrigated twice a week using a drip irrigation system that supplied 17 mm of water per irrigation from planting to 2–3 weeks before anthesis at Manga, while at Kadawa, a furrow irrigation system supplied the same amount of water from planting to 2–3 weeks before anthesis when the irrigation water was withdrawn at each test location. A follow-up rescue irrigation was employed 2 weeks after the withdrawal of irrigation water at both test locations and resumed 10–12 days after the “rescue irrigation” until physiological maturity. The rescue irrigation was applied based on the assessment of some physiological stress indicator traits such as severe leaf rolling during the early mornings, leaf senescence, tassel blasting and leaf wilting. Basal fertilizer rate of 60 kg each of N, P₂O₅ and K₂O ha^{−1} of N, P, K (15:15:15) was applied followed by top dressing 2 weeks after planting (WAP) using 60 kg ha^{−1} of urea. Weed management was carried out using the post emergence herbicide (Roundup) and the pre-emergence herbicides (Caliherb 720 SL and Atrazine) in Ghana, while atrazine and gramoxone were applied in Nigeria at 5 l ha^{−1} followed by manual weeding to ensure weed-free trials.



flowering Days

Figure 1. Mean temperature record for April 2018 and 2019 flowering days at Kadawa.



Flowering Days

Figure 2. Mean temperature record for April 2018 and 2019 flowering days at Manga.

Table 2. Mean monthly rainfall and temperature (day & night) recorded at Manga and Kadawa during the 2018 and 2019 experimental period.

Kadawa		2018			2019		
Month	Day °C	Night °C	Rainfall (mm)	Day °C	Night °C	Rainfall (mm)	
February	36.0	17.0	0.0	31.0	18.0	0.0	
March	38.3	19.4	0.0	37.0	24.0	0.0	
April	40.0	24.0	0.0	39.4	26.2	0.0	
May	39.0	26.0	18.0	37.0	26.0	37.0	
June	37.1	25.0	47.0	34.1	24.1	36.0	
July	34.3	23.0	99.0	31.1	23.0	407.0	

Manga		2018			2019		
Month	Day °C	Night °C	Rainfall(mm)	Day °C	Night °C	Rainfall (mm)	
February	38.2	23.0	0.0	37.1	20.3	0.0	
March	40.0	25.4	0.0	40.5	25.3	0.0	
April	39.4	26.1	18.2	40.1	28.0	40.4	
May	38.0	25.0	7.9	37.0	26.2	12.8	
June	34.0	24.0	15.0	33.4	24.4	15.6	
July	31.4	23.0	16.5	32.0	23.0	17.5	

2.5. Data Collection

Agronomic data recorded under combined heat and drought stress at both Kadawa and Manga testing sites were similar to that described by Nelimor et al. [16]. We measured days to anthesis (AD) as the number of days from planting to when 50% of the plants in a plot had extruded pollen, and days to silking (SD) as when 50% of the plants had produced silks. The anthesis–silking interval (ASI) was recorded as the difference between SD and AD. Plant and ear heights were measured at about three weeks after flowering. Leaf death (LD) was determined by estimating the percentage of leaves dead from the base of the plant to the flag leaf using a scale of 1–9 where 1 = 1% to 10% of leaves dead and 9 = 81% to 100% of leaves dead at 70 days after planting (DAP). At about three weeks post-flowering, data were collected on husk cover (HUSKC), plant aspect (PLASP), plant height (PLTH), ear height (EHT), leaf firing and tassel blasting (TB), as described by Badu-Apraku and Fakorede [22]. A few days prior to harvesting, data were recorded on root and stalk lodging and number of plants per plot. At harvesting, records were taken on ear aspect and number of ears per plant (EPP). EPP was obtained by dividing the total number of ears per plot by the number of plants per plot. For the experiments conducted under optimal environments, the moisture content of the grains at harvest was recorded for the shelled grains of five randomly selected ears per plot using a moisture meter. Grain yield (kg ha^{-1}) was estimated from field weight of ears per plot, assuming a shelling percentage of 80, adjusted to moisture content of 15%. For the trials conducted under combined HD stress, grain weight on plot basis was obtained by shelling all harvested ears of each plot.

2.6. Statistical Analysis

The data were subjected to analysis of variance employing PROCGLM of SAS as well as the RANDOM statement with a TEST option [36] for grain yield and other measured traits. In the statistical model, environments, replicates within environments and incomplete blocks within replicates $\times$ environment interaction were considered as random factors, while the inbred lines were regarded as a fixed factor. Block effects on genotype means were adjusted using the lattice design proposed by Cochran and Cox [37]. Mean values of measured characters were separated using the standard error of difference (SED). The multiple trait selection index was adopted for the identification

of inbred lines combining tolerance to drought and heat stress environments. The base index was computed using Yield, EPP, ASI, PLASP, EASP and LD [38] as follows:

$$MI = [(2 \times \text{Yield} + \text{EPP}) - \text{ASI} - \text{PLASP} - \text{EASP} - \text{LD}] \quad (1)$$

Mean values of the six measured traits were standardized prior to calculation of the MI as the traits were measured in different units. Furthermore, estimated means of the different traits of 15 best and 10 worst inbred lines were adopted for the Genotype $\times$ Trait (GT) biplot analysis [39,40] to obtain information on the secondary traits which could be relied on for indirect selection for combined HD stress. The polygon and the vector views of the GT biplot were constructed using the measured characters and the genotype-focused singular value partition ('SVP = 2'), rendering the polygon view suitable for visualizing the relationship between traits.

3. Results

3.1. Summary Statistics

The summary statistics from the 9684 DArT SNP markers is presented in Table 3. Heterozygosity values were in the range of 0.00 to 0.20 with a mean value of 0.08. The 9684 DArT SNP markers had a major allele frequency ranging from 0.50 to 0.95 with an average value of 0.79. Gene diversity (GD) values varied from 0.09 to 0.50 with a mean of 0.30. For this set of inbred lines, the polymorphic information content (PIC) varied from 0.08 to 0.38 with a mean value of 0.25 (Table 3). The minor allele frequencies were (MAF) 0.05 for minimum and 0.50 for maximum, with an average of 0.21.

Table 3. Diversity statistics of the 162 maize inbred lines based on 9684 DArT single nucleotide polymorphism (SNP) markers.

	MaF	GD	He	PIC	MAF
Minimum	0.50	0.09	0.00	0.08	0.05
Maximum	0.95	0.50	0.20	0.38	0.50
Mean	0.79	0.30	0.08	0.25	0.21

MaF = Major allele frequency, GD = Gene diversity, He = Heterozygosity, PIC = Polymorphic information content, MAF = Minor allele frequency.

3.2. Genetic Distance and Population Structure Using DArT SNP Markers

From the pairwise comparisons of the inbred lines, the genetic distance for the lines was in the range of 0.02 to 0.48, with a mean value of 0.42. About 70.32% of the genetic distance among the inbred lines fell within 0.416 and 0.461 (Figure 3). Lines TZdEI 134 and TZdEI 198 recorded the least genetic distance (Supplementary Table S1), consistent with their genetic background as they shared similar ancestry and history of selection (Supplementary Table S2). Inbred lines TZEI 500 and TZEI 7 recorded the highest (0.48) genetic distance, indicating that the two inbred lines diverged significantly. TZEI 500 was developed from the bi-parental cross involving TZEI 11 $\times$ TZEI 8 through repeated selfing up to the 8th generation of inbreeding, while TZEI 7 was developed from repeated self-pollination of WEC STR up to the 8th generation. Moreover, TZEI 500 is characterized by yellow endosperm while TZEI 7 has white endosperm (Supplementary Table S2).

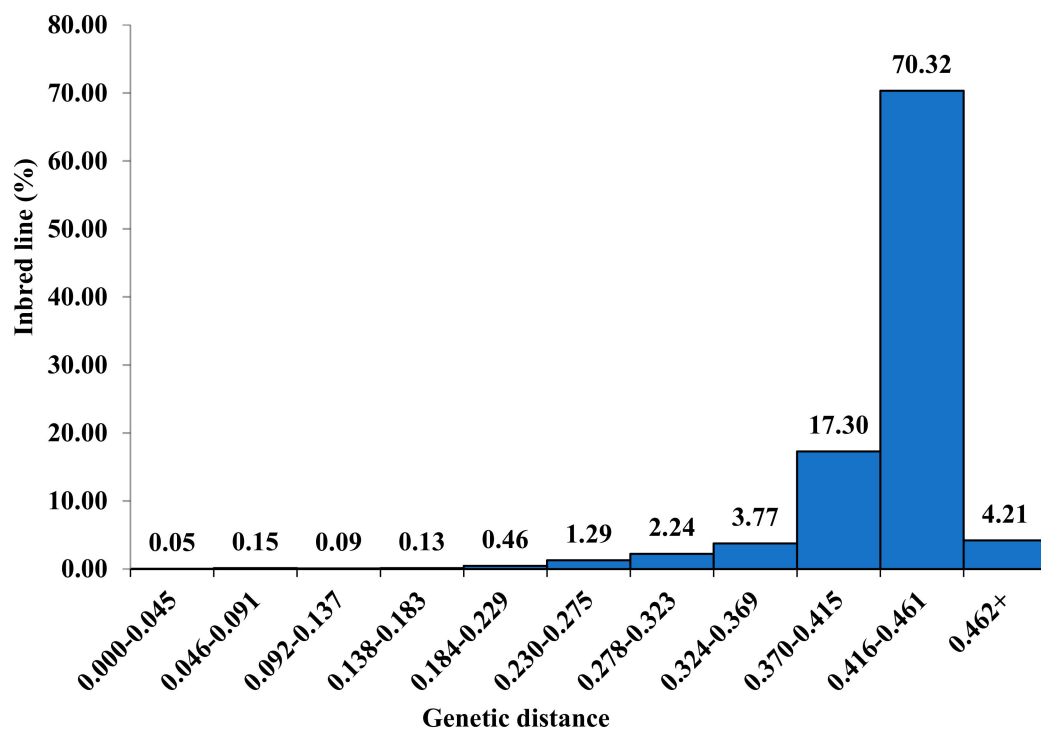


Figure 3. Frequency distribution of genetic distances based on Euclidean method for 162 early yellow and white inbred lines genotyped using 9864 DArT SNPs.

The population structure analysis of the 162 early white and yellow tropical maize inbred lines using the selected 9684 SNPs revealed an optimal k of six sub-populations (Figure 4A). Sub-population 1 comprised 8.02% (13 of the inbred lines), 16.67% (27 of the inbred lines in sub-population 2) and 8.64% (14 of the inbred lines in sub-population 3), while sub-populations 4, 5 and 6 contained 3.09% (5 lines), 6.17% (10 lines) and 4.32% (7 inbred lines), respectively (Supplementary Table S2). About 53.09% (86 of the inbreds) with the probability of association of less than 70% were grouped as mixed individuals (Supplementary Table S2). These six sub-populations were separated based largely on the pedigrees and endosperm colours. Sub-populations 1, 2, 4, 5 and 6 consisted of white endosperm maize inbred lines while sub-population 3 comprised yellow endosperm lines, with about 53% consisting of a mixture of white and yellow endosperm colour (Supplementary Table S2). The results showed that subpopulations 2, 3 and 4 contained only an inbred line, each derived from the different source populations: TZEI 11 $\times$ TZEI 8 S₈, TZE COMP 5-W DT C7 $\times$ TZEI 65 S₈ and TZE COMP 3 DT C₂ F₂ $\times$ TZEI 98 S₈, respectively. Sub-population 5 consisted of two source populations with inbred lines derived from TZE W- P_{OP} STR 108 S₈ and TZE -W P_{OP} STR 104 S₈. Sub-population 1 consisted of four dissimilar source populations, which included TZE COMP 5 W DT C7 $\times$ TZEI 2, (TZEI 7 $\times$ TZEI 3) S₈, TZE - Y P_{OP} STR 106 S₅, and TZE COMP 5 -W DT C7 $\times$ TZEI 31 S₈. Sub-population 6 had the highest genetic diversity and comprised five source populations, namely WEC STR S₈, TZE-W P_{OP} Co S₈, TZE -W P_{OP} $\times$ LD S₈, TZE COMP₃ DT C₂ F₂ $\times$ TZEI 60 S₈, TZE-Y P_{OP} STR 106 S₈ and TZE W P_{OP} STR 107 S₈.

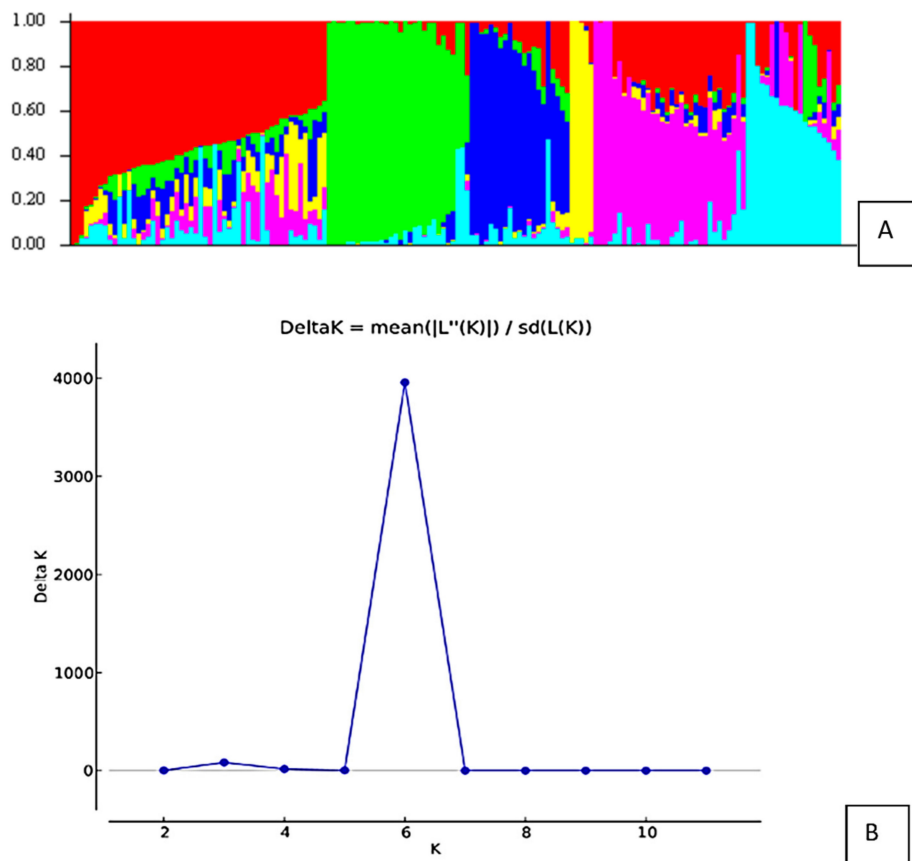


Figure 4. The six sub-populations (1 = red, 2 = green, 3 = blue, 4 = yellow, 5 = pink and 6 = teal) of the 162 early maturing yellow and white inbred lines using DArT SNP markers. (A) population structure bar plot of the 162 early yellow and white inbred lines as shown by 9684 DArT SNP markers for $K = 6$. (B) Assessment of the best delta K by the Evanno method.

Within the six sub-populations, the expected heterozygosity amongst the inbred lines ranged from 0.10 for sub-population 4 to 0.43 for sub-population 6 with a mean of 0.25 (Supplementary Table S3a). Overall, sub-populations 4 and 6 recorded the highest allele frequency (0.23), while sub-populations 1 and 5 recorded the least allele frequency (0.09) (Supplementary Table S3b). For each of the sub-populations, the STRUCTURE analysis estimated the fixation index (F_{ST}) and indicated significant divergence within the six sub-populations. Estimated F_{ST} values of 0.34, 0.49, 0.47, 0.81, 0.45 and 0.28 were recorded for sub-populations 1–6, respectively (Supplementary Table S3c).

3.3. Cluster Analysis and Principal Coordinate Analysis (PCoA)

The neighbour joining (NJ) phylogenetic tree constructed assigned the 162 inbred lines into three main clusters followed by several sub-clusters (Figure 5). In conformity with the biplot output of the STRUCTURE analysis (Figure 4A,B), the six clusters could be clearly depicted in the phylogenetic tree (Figure 5). Unique colours were used to represent each sub-population with red, green, blue, yellow, pink and teal denoting 1–6, respectively, and the black colour represented the mixed population when 70% probability threshold was considered.

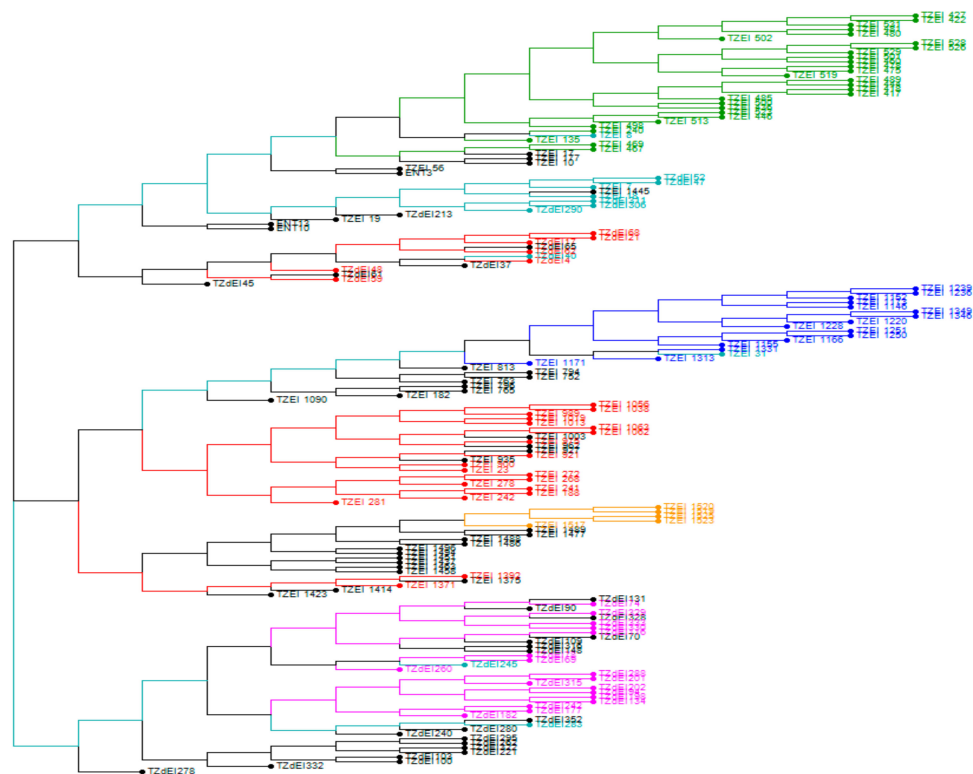


Figure 5. Clustering of 162 yellow and white early tropical maize inbred lines based on 9684 DaRT SNP markers (red (cluster 1), green (cluster 2), blue (cluster 3), yellow (cluster 4), pink (cluster 5), teal (cluster 6) and black (admixture)).

Based on the phylogenetic tree, a total of 27 inbred lines were placed in cluster 1, with 13 out of the 27 inbreds derived from six different white maize backgrounds, TZE-W Pop STR Co S6, (TZEI 7 $\times$ TZEI 3) S6, (TZE COMP 5-W DT C7 $\times$ TZEI 31) S6, (TZE COMP 5-W DT C7 $\times$ TZEI 2) S6 and TZEE-Y POP STR 106 S5 189/194, representing sub-population 1 (red bars; Figure 5). The second cluster included 29 inbred lines, of which 27 clearly represented sub-population 2 (green bars). All the inbred lines in this cluster were derived from the yellow endosperm bi-parental cross (TZEI 11 $\times$ TZEI 8) S₈ (Supplementary Table S2). The third cluster consisted of 16 inbred lines with 14 being similar to those generated from the population structure analysis (sub-population 3; blue bars). All the inbred lines from this cluster were derived from one white kernel source population (TZE COMP 5-W DT C₇ $\times$ TZEI 65) S₈. Cluster 4 had 23 inbred lines out of which 5 were derived from the white endosperm population, (TZE COMP 3 DT C₂ F₂ $\times$ TZEI 98) S₈, and were similar to those generated from the population structure (sub-population 4; orange bars). The fifth cluster obtained from the phylogenetic analysis comprised a total of 18 inbred lines, with 10 out of these belonging to the white endosperm maize population, TZE-W Pop STR 104 S₅ (sub-population 5; pink colour). The results of the grouping of the 10 inbreds lines into sub-population 5 was consistent with the results obtained from the population structure analysis. The remaining eight inbred lines of the fifth cluster were derived from the source population, TZE-W Pop STR 108, and were also characterized by white endosperm kernels. The sixth cluster consisted of 12 inbred lines, with seven of them similar to those generated from the population structure analysis (indicated with teal colour lines) and represented by sub-population 6. Inbred lines placed in this cluster were derived from six different source populations with each inbred line extracted from a different source population, with the exception of the inbred lines TZdEI 47 and TZdEI 52 derived from the same source population, TZE-Y Pop STR 106 (Supplementary Table S2).

To understand and confirm the dynamics of the inbred population structure, we also adopted the principal coordinate analysis (PCoA). Based on the pairwise genetic distance matrix among the

162 inbred lines, a clear distinction among the six groups of inbred lines could be visualized, and this was in concordance with the results of the population STRUCTURE biplot (Figure 6).

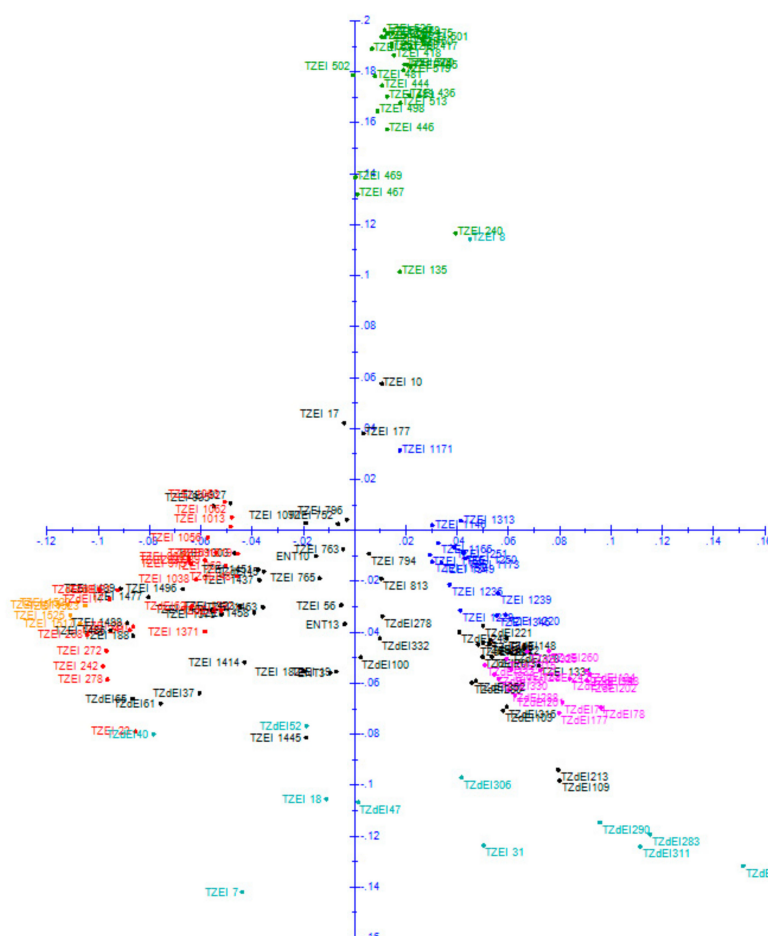


Figure 6. Principal coordinate analysis (PCoA) of 162 early yellow and white inbred lines genotyped using 9864 DArT SNPs.

3.4. Genotype-by-Trait (GT) Interaction and the Multiple Trait Selection Index

The GT biplot analysis is one of the most reliable statistical tools available for selecting inbred lines for hybrid development using secondary traits. This analysis can identify secondary traits which are directly or indirectly related to traits of major breeding interest such as grain yield and ears per plant. The inbred lines were evaluated under combined heat and drought stress in this study to select desirable seed parents that could be used in productive hybrid combinations. Moreover, the evaluation aimed to examine the inter-relationships among traits measured for the inbreds as a way of predicting outstanding hybrid performance. The present study identified 15 inbred lines with outstanding performance as well as those with poorest performance (worst 10 lines) under combined heat and drought environments using the GT biplot (Figure 7). The inbred lines were selected based on the premise that the best selected lines should not record 50% or more percentage count for leaf firing and tassel blasting under combined HD stress. Approximately 52.56% of the total variation among the measured traits of the inbred lines was accounted for by the principal component axes PCI and PC2. In the biplot view, high values of grain yield and EPP for inbreds were considered as desirable while high values for the secondary traits such as LF, TB, EASP, PLASP, LD, ASI, HUSKC, AD, SD, and SL of inbreds were considered undesirable. The inbred(s) at each vertex of the polygon (vertex inbred) had the highest values for the traits within its sector. The results revealed that the sector with inbreds 21 and

8 as the vertex inbreds contained the traits LD, TB, LF, ASI, HUSKC, SL, EASP and PLASP, indicating that these inbreds had high values for these traits and that they were the low-yielding inbreds based on the high values of these traits. Inbred lines 25 and 20 were positioned at the vertex that contained the RL traits, signifying that these inbreds recorded high values for root lodging. In contrast, inbred 1 (the best performing inbred line) was the vertex inbred in the segment containing EPP and grain yield, suggesting the superiority of the inbred line in terms of grain yield and EPP. Inbred line 7 was the vertex inbred that recorded no trait within its sector, implying that it was not outstanding in any of the measured traits. Fifteen inbred lines were identified as outstanding in performance based on the measured traits. TZEI 935 was identified as the best performing inbred line with a yield of 3461 kg/ha. Contrarily, 10 inbred lines were identified as the worst in performance based on the measured traits, with TZdEI 109 as the worst performing inbred line with a yield of 424 kg/ha (Table 4).

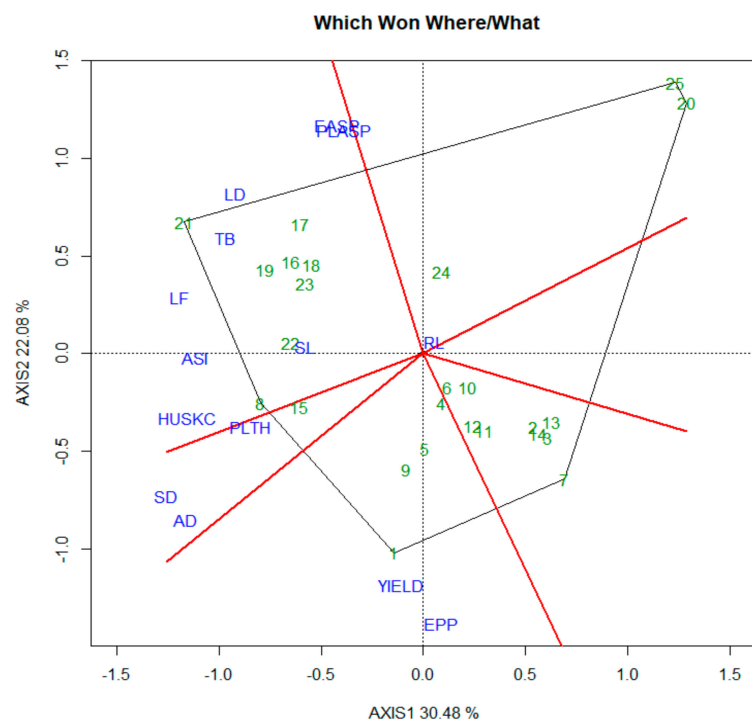


Figure 7. A “Which is best/worst for what” or “Which wins where” of GxT biplot of the 25 early maturing maize inbreds assessed under combined heat and drought conditions at Kadawa in Nigeria and Manga in Ghana during the dry seasons of 2018 and 2019. PC1 and PC2 for model 2 explained 52.56% of the variation among traits. YIELD = yield, EPP = ears per plant, RL = root lodging, SL = stalk lodging, SD = days to silking, AD = days to anthesis, ASI = anthesis silking interval, PLTH = plant height, LF = leaf firing, TB = tassel blast, LD = leaf death, HUSKC = husk cover, EASP = ear aspect, PLASP = plant aspect.

Table 4. Mean grain yield and other agronomic characters and codes of 25 selected inbred lines evaluated under combined heat and drought environments at Manga-Ghana and Kadawa-Nigeria during the 2018 and 2019 dry seasons.

Code	Entry	Yield (ka/ha)	EPP	AD	SD	ASI	RL	HUSKC	PLASP	PLTH	SL	EASP	LD	LF	TB	M.I
1	TZEI 935	3461.12	1.83	58.72	61.61	2.89	0.67	3.68	4.58	90.37	1.51	4.99	2.81	0.03	0.02	12.96
2	TZEI 1019	1447.28	1.43	54.07	56.51	2.40	0.66	2.96	4.06	92.66	1.46	3.96	2.84	0.04	0.01	9.68
3	TZEI 240	1384.51	0.90	55.53	57.71	2.14	0.34	3.00	3.75	97.93	1.14	3.92	2.25	0.03	0.00	8.82
4	TZEI 56	1833.74	0.87	57.36	59.76	2.41	0.31	3.54	4.53	94.43	0.82	4.10	2.86	0.05	0.09	7.04
5	TdZEI 295	1273.46	0.98	58.95	61.60	2.65	1.03	3.38	4.03	90.21	1.03	3.62	2.61	0.18	0.07	6.72
6	TdZEI 311	834.04	0.75	56.85	58.44	1.57	0.85	4.02	4.08	112.35	1.32	3.90	2.79	0.07	0.05	6.03
7	TZEI 1228	1002.42	0.86	55.91	58.49	2.58	0.86	3.21	2.88	85.49	0.17	3.39	2.27	0.05	0.02	5.72
8	TdZEI 278	1420.57	1.09	58.33	61.74	3.41	1.17	3.76	3.97	97.71	1.85	3.52	3.41	0.37	0.42	5.52
9	TZEI 478	1572.27	1.09	57.89	61.27	3.37	0.61	3.25	3.93	104.35	0.50	3.52	2.91	0.24	0.00	5.48
10	TZEI 475	1061.64	0.80	56.29	57.77	1.52	0.37	3.46	4.02	94.07	2.37	3.47	2.97	0.15	0.02	5.26
11	TZEI 135	1216.49	0.81	56.69	58.64	1.94	2.61	3.76	3.87	97.14	1.59	3.63	2.50	0.07	0.01	5.22
12	TZEI 763	1195.72	0.86	59.19	61.52	2.34	0.23	3.87	4.38	74.40	0.32	4.24	2.23	0.02	0.03	4.85
13	TZEI 23	854.59	0.76	55.57	57.80	2.22	0.48	3.08	3.26	78.24	1.75	3.38	2.54	0.03	0.02	4.82
14	TZEI 17	1041.86	0.98	57.51	59.17	1.67	0.84	3.59	3.80	80.81	0.33	4.48	2.04	0.07	0.01	4.73
15	TdZEI 69	1261.13	0.99	56.74	59.23	2.49	1.47	3.96	4.45	155.73	1.37	4.08	2.71	0.48	0.09	4.72
16	TZEI 278	828.89	0.54	59.30	61.68	2.42	0.35	4.73	5.01	90.07	1.48	5.49	3.57	0.17	0.01	−5.26
17	TZEI 31	699.92	0.44	57.71	60.51	2.81	0.98	3.84	4.70	85.13	2.21	4.92	3.56	0.23	0.28	−5.29
18	TdZEI 280	820.45	0.56	58.30	61.33	3.03	0.43	3.93	4.83	97.40	1.39	5.34	3.10	0.20	0.19	−5.33
19	TZEI 427	931.83	0.52	57.80	61.88	4.10	0.19	3.76	4.37	92.45	1.57	5.28	3.01	0.30	0.36	−5.42
20	TZEI 502	493.36	0.36	48.93	50.01	1.08	1.43	2.18	4.70	62.69	0.66	5.44	2.80	0.07	0.12	−5.50
21	TZEI 1003	698.00	0.45	58.36	61.88	3.51	0.28	3.65	4.74	114.21	1.85	5.10	3.10	0.51	0.57	−5.50
22	TdZEI 306	845.25	0.59	61.13	65.04	3.92	0.56	3.85	4.19	90.09	0.61	4.95	3.59	0.15	0.02	−5.66
23	TdZEI 61	722.60	0.52	58.06	62.21	4.15	2.77	3.60	4.33	106.77	0.62	5.01	3.43	0.21	0.13	−6.46
24	TZEI 1349	436.40	0.39	54.69	58.80	4.09	0.54	3.82	4.41	94.67	−0.03	5.00	2.64	0.06	0.01	−6.71
25	TdZEI 109	423.70	0.36	47.42	48.89	1.47	0.79	2.48	5.34	74.93	0.76	4.97	3.11	0.02	0.00	−7.51
Means		1110.45	0.79	56.69	59.34	2.65	0.83	3.53	4.25	94.17	1.15	4.39	2.87	0.15	0.10	

EPP = ear per plant, RL = root lodging, SL = stalk lodging, SD = days to silking, AD = days to silking, ASI = anthesis silking interval, PLTH = plant height, LF = leaf firing, TB = tassel blast, LD = leaf death, HUSKC = husk cover, EASP = ear aspect, PLASP = plant aspect, M.I = multiple trait base index.

4. Discussion

Molecular markers have proven to be among the most reliable tools for identifying the genetic components of inbred lines [15,29]. In the present study, 9684 DArT-based SNP markers were used to assess the genetic diversity of 162 early maturing yellow and white endosperm tropical maize inbreds possessing varying degrees of tolerance to drought and/or combined HD stress. Moreover, the population structure, heterotic groups and inter-trait relationships were also examined in the studied materials.

Generally, high PIC values indicate the effectiveness of markers for linkage analysis when estimating the inheritance between parental lines and the derived hybrids [41], whereas the GD or expected heterozygosity (H_e) reveals gene diversity for haploid markers and gives information on the proportion of the genetic distance and average heterozygosity within a given population [41,42]. In the present study, an average PIC of 0.25 was obtained, which is comparable to that reported earlier by Dao et al. [27] and Zhang et al. [28], as well as the 0.24 of Yu et al. [43] and 0.29 of Wu et al. [44], but higher compared with the results of Adu-Boakyewaa et al. [15] and Silva et al. [45] who reported average PIC values of 0.19 and 0.17, respectively. The high mean PIC value observed implied that majority of the selected SNPs were informative and polymorphic enough to bring out the differences among the 162 inbred lines studied. The average GD of 0.30 obtained in this study is also comparable to 0.32 previously reported in maize [46,47], but higher than the value (0.22) reported by other researchers [15,44,48,49]. These results indicated a broad genetic variability among the inbred lines and the relatively lower number of pairwise individuals with low genetic distance.

Of the 9684 SNPs deployed across the 162 lines, approximately 65% had MAF of between 0.05 and 0.25 (Supplementary Table S2), which is lower compared to the range reported by Semagn et al. [17] involving 450 CIMMYT lines studied using 1065 SNPs. Nevertheless, it is comparable to the values obtained by Adewale et al. [19] in early maturing white tropical maize. Consistent with the results of Adu-Boakyewaa et al. [15] and Adewale et al. [29], the average heterozygosity of 0.08 obtained in the present study is close to zero as expected for inbred lines, thus making them a useful genetic resource for association mapping and genetic studies in maize in which uniformity of the genetic materials is invaluable.

A higher F_{ST} value for the sub-populations was reported in the present study. Together with the relatively moderate allele frequency divergence among sub-populations, the results indicated that the alleles of the inbred lines were fixed and could be categorized into groups with distinct characteristics, and this finding is in conformity with previous results [15]. The six clusters observed in this study indicated that individuals from the different groups were therefore expected to harbour different favourable alleles that could be exploited for breeding for combined HD-tolerant hybrids and synthetics. The results also demonstrated that the DArT-based SNP-derived markers were informative in providing genome profiles which were very useful for the identification of unique characteristics among the inbred lines [17,50,51].

Population structure analysis provides a guide for assigning a set of inbred lines into heterotic groups based on genotypic information of individual ancestry of the lines [52]. In this study, six sub-populations ($K = 6$) were established among the 162 inbred lines. The inbred lines were placed in distinct groups based on their relatedness in terms of pedigree records and selection history, such as endosperm colour, tolerance or susceptibility to drought and combined HD. This finding was affirmed by the high F_{ST} values ranging from 0.28 to 0.81 for the six sub-populations and that inbred lines from the different groups could harbour unique favourable alleles for development of productive hybrids for population improvement programmes in the tropics. The high F_{ST} values obtained for the sub-populations in the present study were comparable to the results of other researchers. For example, Kashiani et al. [53], Aci et al. [54] and Adu-Boakyewaa et al. [15] indicated F_{ST} values of as high as 0.94, 0.33 and 0.83, respectively, for the sub-populations obtained in their genetic diversity studies for different sets of tropical maize lines. In the present study, cluster 1 contained all the inbred lines with white endosperm and were generally tolerant to drought, cluster 2 had inbred lines with

yellow endosperm as well as tolerance to drought, while cluster 3 had highly drought-tolerant white endosperm lines. However, all the inbred lines within these three sub-groups showed moderate tolerance to combined HD stress. This observation is not surprising because the inbred lines have long been selected under drought conditions compared to the results of the more recent evaluations under combined HD conditions.

The multivariate clustering methods showed high consistency regarding the number of groups and individuals assigned to each group, indicating the existence of six genetically distinct groups. This result corroborated the findings of Adu-Boakyewaa et al. [15] and Obeng-Bio et al. [51] who reported a high consistency among the model-based structure analysis, PCoA and the Neighbour-Joining phylogeny using the Roger's and Euclidian genetic distance methods, respectively. However, this result disagreed with the reports of Dao et al. [27] and Semagn et al. [17] who found a high consistency between the model-based structure and the PCoA but very low concordance with the Neighbour-Joining phylogeny generated using the Roger's genetic distance method. The differences in the results could be due to the variation in the inbred lines used in the different studies, the method used in deriving the genetic distances between the inbreds, as well as the different clustering algorithms used. In concordance with earlier researchers, it was evident that the clustering of tropical maize populations is largely consistent with the pedigree information [15,21,27,48,55].

Generally, it is advocated that source genotypes with favourable alleles for combined heat and drought tolerance should possess good agronomic characteristics necessary for combined HD-tolerant hybrid development without placing too much emphasis on the *per se* grain yield. The GT analysis identified ASI, EASP, PLASP, LF, TB and EPP as important secondary traits that could be utilized to accelerate genetic gains from selection for enhanced grain yield under combined HD conditions. Nelimor et al. [16] suggested that lines that scored 50% or more for LF and TB under combined HD conditions should be rejected, implying the importance of the information on inter-relationships among secondary traits. From the GT biplot view, the best 15 performing lines were placed in five different heterotic groups. As a guide, crosses among the lines from different heterotic groups should be given priority in terms of hybrid combinations towards maximization of heterosis. For example, the best performing inbred line (TZEI 935) placed in the mixed group could be crossed to inbred lines TdZEI 311 and TZEI 1228 which belonged to groups six and three, respectively, to increase the chances of developing productive hybrids. The significant and positive association observed between PLASP and EASP implied that there was a close relationship among these traits and that either of them could be eliminated from the base index without sacrificing the precision in selecting for combined HD-tolerant genotypes. Badu-Apraku et al. [38] also found a positive correlation between these traits and advocated that either PLASP or EASP could be discarded in the base index in selecting for drought- and low-N-tolerant genotypes. Similarly, there was high positive correlation between AD, SD, and ASI, but none of them were found to be redundant as the ASI is dependent on the SD and AD [38]. These three traits were very important in selecting stress-tolerant maize genotypes [56,57], as a relatively constant value (lower ASI) is desirable in selection of early maturing maize [38].

5. Conclusions

To ensure the selection of appropriate parental lines for crossing and devising strategies for significant gains from selection, adequate knowledge and understanding of the genetic diversity of available inbred lines in a breeding programme is essential. The population structure analysis, PCoA and phylogenetic clustering methods confirmed the existence of six different groups for the panel of 162 maize inbred lines studied. The lines were grouped largely based on the pedigree information, endosperm colour, selection history and similarity of ancestry. There were high genetic distances between the paired inbred lines, demonstrating the distinctiveness of the inbred lines and the presence of high genetic variability which could be exploited in tropical maize breeding programmes for hybrid development. The low heterozygosity as well as relatively low divergence among the sub-populations indicated that the present set of inbred lines had great potential for genetic studies and could serve as

future sources of promising inbred lines by contributing new and favourable alleles. The GT biplot analysis revealed that EPP, PLASP, EASP, LF and TB were the most reliable traits for selecting genotypes for tolerance to combined HD stress. Inbred 1 (TZEI 935) was identified as the ideal under combined HD stress environments. Using the base index, the 15 top-yielding inbred lines were identified for use in the maize breeding programmes of SSA for the development of productive hybrids and synthetics with combined HD tolerance. Among the inbred lines studied, inbred TZEI 935 was identified as one of the ideal lines under combined HD stress environments and should be further explored. Finally, the results of the present study will serve as an important guide for appropriate decision-making for future development of productive hybrids, particularly for SSA.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4395/10/9/1324/s1>, Table S1. Genetic distance matrix of the 162 yellow and white inbred lines using Euclidean method in the present study. Table S2. Summary of the 9684 SNP markers used in the current study. Table S3. a. Expected Heterozygosity of the 162 yellow and white inbred lines; b. Allele frequency divergence among populations with 162 inbred lines assessed and c. mean values of Fixation Index (F_{ST}) of 162 yellow and white inbred lines.

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Conflicts of Interest: The authors declare no conflict of interest.

Data Availability: The DARtseq datasets used in the present study have been deposited at the IITA CKAN repository.

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