IDENTIFICATION OF HEAT TOLERANT LINES AND GENETIC DIVERSITY IN WHEAT (*Triticumaestivum*L.) USING MOLECULAR MARKERS IN GUINEA SAVANNA, NIGERIA

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Abstract: Field trials were conducted at Lake Chad Research Institute Research Farm at Dadinkowa, Gombe State-Nigeria, during 2014/2015 and 2015/2016 dry seasons. The objectives of this study was undertaken to identify and evaluate heat tolerant lines and genetic diversity from bread wheat and to explore the potential of molecular markers in improving wheat heat tolerance. 24 wheat lines were used; heat stress was imposed through staggered sowing. Normal sowing (15th November) was non-stress and late sowing (6th January) resulted in terminal heat stress. The lines were laid out in Randomized Complete Block Design (RCBD) in triplicates during 2015/2016 dry season in plots measuring 3x2 m with 6 rows and 30 cm row spacing. The analysis of variance was computed using the General Linear Model (GLM) SAS version 9.2. Wizard Genomic DNA Purification Kit was used for DNA extraction. DNA was extracted by Cetyltrimethylammonium Bromide (CTAB) method. The result of means square indicated significant difference between genotypes. The results also indicated that the number of alleles range from 1- (Dreb-B1) to 9- (Xgwm577), genetic diversity index varied greatly among the loci from 0.0000 in case of Dreb-B1 to 0.8471 in case of Xgwm577. The Polymorphic Information Content (PIC) value were from 0.0000 (Dreb-B1) to 0.8296 (Xgwm577). The lowest and highest genetic distances level were 0.083 and 0.750, respectively. Cluster analysis had grouped the accessions into 5 groups at a genetic distance level of 0.15. In conclusion, genotypes 4404, 4408, 4410, 4411, 4413, 4414, and 4420 were identified as top yielder as such could be explored for resistance lines against heat stress.

Keywords: Diversity, DNA, genetic, heat, marker, QTL, wheat

Introduction

Wheat (*Triticumaestivum* L. emThell.) is the first important and strategic cereal crop for the majority of world's populations. The grass family Poaceae includes major crop plants such as wheat, barley (*Hordeumvulgare* L.), oat (*Avena sativa* L.), rye (*Secalecereale*L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.). *Triticeae*is one of the tribes containing more than 15 genera and 300 species including wheat and barley. World wheat production was estimated at 734.51 million metric tons (USDA, 2015). In Nigeria, domestic wheat production was low and stood at 70,000 metric tons in market year 2013/2014 (USDA, 2015). Nigeria's northern states of Borno, Yobe, Jigawa, Kano, Zamfara, Katsina, Adamawa, Sokoto and Kebbi, are major wheat growing areas. Boko Haram insurgencies are growing stronger in many of these areas and therefore, ocal wheat production has declined during these years due the insecurity, unfavorable climatic conditions, and expensive cost of production (USDA, 2015). The crop is cultivated under irrigation during the cold "Harmattan" period between November and February, which provides the required low night temperatures ranging from 10 to 25ºC (Abbas, 1988).

Heat stress severely restricts wheat growth and productivity and is considered as one of the major abiotic adversities for many crops (Boyer, 1982; Georgieva, 1999; Hassan, 2006) particularly when it occurs during reproductive stages, which may lead to substantial yield loss in wheat (Hays *et al.,* 2007). The rising temperatures of the late phases of wheat development and particularly, from the beginning of heading and after anthesis, should be considered as an important factor limiting yield (Macas *et al.,* 1999a, 2000b; Dias and Lidon, 2009).

In breeding programs, it is desirable to have large genetic diversity for the creation of new genotypes. The aim is to measure the genetic similarity (GS) and genetic distance (GD) among parents, which can be used to estimate the expected genetic variation in different combinations of progeny. In general, the study of genetic diversity has two major objectives: analysis of the levels of polymorphism among certain individuals and studies of the distribution of polymorphism (Kremera *et al.,* 1998). Genetic diversity can be assessed form pedigree analysis, morphological traits or using molecular markers and it is the material basis for crop improvement (Habash*et al.,* 2009). DNA markers are technology that can increase breeding progress, especially for traits that are controlled by multiple genes which are difficult to select under field conditions (Habash*et al.,* 2009). Microsatellites are repeating sequences of 2–6 base pairs of DNA Simple sequence repeats (SSRs) and are among the most stable markers of genetic variation and divergence among wheat genotypes because they are multiallelic, chromosome-specific and evenly distributed along chromosomes (Roeder *et al.,* 1998). Microsatellite genotyping is used for; genetic biodiversity, population genetics at the level of relatedness, genome mapping, as markers for pathogens, etc. Hypothesis is that the relationship of parents affects the genetic diversity.

Materials and Method

Experimental sites and screening of plant materials

Field trials were conducted at Lake Chad Research Institute (LCRI) Wheat Research Farm at Dadinkowa, Gombe State-Nigeria. Dadinkowa is located in the Guinea Savanna between latitude 10^0 8^1 N and longitude 11^0 20^1 S on an altitude of about 600 m above sea level. Twenty four (24) lines (Table 1) were used in this study, normal sowing (15th November) sowing was non-stressed; heat stress was imposed through staggered sowing (6th January) resulted in terminal heat stress. The lines were laid out in Randomized Complete Block Design (RCBD) replicated three times in a plots measuring 3x2 m^2 with 6 rows and row spaced at 30 cm apart during 2014/2015 and 2015/2016 dry seasons respectively. Mineral fertilizers were applied at the rate of 120 kg N/ha, 40 kg P_2O_5/ha and 40 kg K_2O/ha, in which all the phosphorus and potassium were applied at planting, while the Nitrogen was

given in three split doses at planting, and at four and eight weeks after planting.

Data on 50% days to heading, 50% days to flowering, number of tillers per plant, plant height (cm), spike length (cm), number of seeds per spike, one thousand-grain weight (g), and grain yield (kg) were recorded. The form of analysis of variance for individual environment was computed using the General Linear Model (GLM) in SAS version 9.2.

DNA extraction and DNA quantification

Wizard Genomic DNA Purification Kit was used for DNA extraction, total genomic DNA was extracted by CTAB method as modified by Udupa *et al.* (1999). Fresh young leaves (30 mg) were collected from individual cultivars. DNA were quantified by use of Agarose Gel Electrophose where each sample was prepared for loading unto Gel as follows; DNA 5 µl, Sterile distilled water 5 µl, and 3 µl of Loading buffer (Agarose blue) Spined down and loaded on to agarose gel. The Gel was prepared by boiling 3.6 g agarose powder in 300 ml of 1x TBE. Electrophoresis was first run at 60V and followed by 80V.

Polymerase chain reaction (amplification and running conditions)

PCR reaction was performed in a reaction volume of 10 µL containing 1x PCR buffer (1.5 mM $MgCl_2$), 200 µM of each dNTPs, 10 pmole of each primer, 0.5 U of Taq DNA polymerase (Promega) and approximately 50 ng of genomic DNA. The PCR products were separated 1.2 or 1.5 % (w/v) agarose gels for functional markers. Linked markers were run in 6 % native polyacrylamide gels, prepared in a vertical electrophoresis unit (CBS Scientific) using 0.5 x TBE buffer. The different gels were stained with ethidium bromide and visualized under UV light.

Molecular data analysis

PowerMarker software version 3.25 (Liu and Muse, 2005) was used to estimate the number of alleles, genetic diversity and Polymorphic information content (PIC) of each locus (Botstein *et al.*, 1980). Genetic distances between each pair of cultivars were measured by estimating the shared allele frequencies (Jin and Chakraborty, 1993). The Neighbor joining dendrogram was generated using the DARwin software based on the genetic distance estimated using PowerMarker software. To measure the in formativeness of the SSR markers, the PIC for each microsatellite were estimated. PIC values were estimated according to Anderson *et al.* (1993) as:

$$\mathbf{PIC} = 1\text{-}\sum_{i=1}^{k} P_i^2$$

Where: k is the total number of alleles detected for a locus of a marker and P_i is the frequency of the i^{th} allele in the set of 24 accessions investigated. The average PIC value is equivalent to the genetic diversity estimated as a measure of genetic variation. Alleles amplified by microsatellite primers for each cultivar were scored and genetic diversity (*H*) was calculated:

$$H = n/(n-1)(1-\sum P^2$$

Where:*n* is the number of samples and *p* is the frequency of an allele.

Table 1: Pedigree and origin of the bread wheat entries used in this study

Entry	Pedigree	Origin
4401	HUBARA-2/QAFZAH-21//DOVIN-2	HTPYT-404
4402	INQALAB 91x2/TUKURU//WHEAR	HTPYT-425
4403	ATILLA50//ATILLA/BCN/3/STARx3/MUSK-3	HTPYT-407
4404	KAUZ/MON/CROWS/3/VEE/PJN//2x/KAUZ	HTPYT-413
4405	HUBARA-16/2xSOMAMA-3/12AP-4AP	HTPYT-422
4406	HUBARA-16/2xSOMAMA-3/5AP-16AP	HTPYT-424
4407	FLORKWA-2/6/SAKER'S'/5/RBS/ANZ/3/KUZ/HYS//	HTPYT-403
4408	ZAKIA-5	HTPYT-417
4409	KAUZ'S'SERI/3/KAUZ//KAUZ/STAR	HTPYT-409
4410	HUBARA-3x2/SHUHA-4	HTPYT-427
4411	NEJMAH-12	HTPYT-416
4412	SAUAL/3/C80.1/3xBATAVIA//2xWBLL1/4/SAUAL#1	HTPYT-209
4413	WBLLI/4/BOW/NKT/CBRD/3/CBRD/5/WBLLIx2…	MX110-11(M45IBWSN-189)
4414	ATILLAx2/PBW65x2/5/BOW/NKT//CBRD/3/CBRD…	MX110-11(M45IBWSN-177)
4415	P1.861/RDWG/4/SERI.B//KAUZ//HEVO/3/AMAD	ICARDA-WIP-173
4416	KAUZ'S'SERI/3/KAUZ//KAUZ/STAR-1	ICARDA-WIP-194
4417	ATTILAx2/PBW65x2/4/BOW/NKT//CBRD/3/CBRD CMSS06Y01026T-099TOPM-099Y-099ZTM-099Y-099M-18WGY	MX110-11(M45IBWSN-184)
4418	KAUZ//MON/CROW/4/SERI.1B//KAUZ/HEVO/3/AMAD	4TH ESBWYT2-302
4419	KACHU#1/4/CROC_1/AE.SQUARROSA(205)//KAUZ/	MX110-11(M45IBWSN-170)
4520	PFAU/WEAVERx2/BRAMBLING/3/KAUZ/TRAP#BOW…	MX110-11(M45IBWSN-193)
4521	ATTILAx2/PBW65x2/4/BOW/NKT//CBRD/3/CBRD CMSS06Y01026T-099TOPM-099Y-099ZTM-099Y-099M-6WGY-.	MX110-11(M45IBWSN-181)
4522	ATILLA50Y//ATILLA/BCN/3/STARx3/MUSK-3	1STWHTON-104
4523	KAUZ/RAYON/3/N5732/HER//CASKOR	1STWHTON-90
4524	KHALIFA	1STWHTON-80

Source: LCRI. The nomenclature described by Skovmand *et al*. (1997) was used for writing pedigrees

Result and Discussion

Mean squares

Table 2 shows mean squares from the analysis of variance for the phenotyphic characters (growth and yield) for normal and heat stressed wheat evaluated in 2015/2016 cropping season. The results indicate significant increase in genotypes for plant height, number of tillers, and spike length at 1% probability level. Furthermore, significant difference were also observed between genotypes for days to 50% heading, days to maturity, and yield at 5% probability level. Similarly, the results for the heat stressed condition indicated significant difference ($P<0.01$) between genotypes for days to 50% flowering and spike length. While, significant increase ($P<0.05$) were also observed between the genotypes for number of tillers and days to 50% maturity. The yield attributes under heat stress is a good indicator of heat tolerant Wahid *et al.* (2007). In this study genotypes 4404, 4408, 4410, 4411, 4413, 4414, and 4420 were identified as top yielders as such could be explored for development of resistant lines against heat stress.

Table 2: Mean squares (MS) from the analysis of variance for phenotyphic characters (growth and yield) under normal and heat stressed conditions in 2015/2016 cropping season

Source of Variation	Degree of Freedom	Stand Count	Days to 50% heading	Days to 50% flowering	Plant height (cm)	Tiller Count	Spike Length (cm)	Seed per Spike	Spikelet per Spike	Day to Maturity	1000 Grain Weight(g)	Yield (kg/plot)
NS												
Rep	2	6.889	7.347	6.500	5.732	1441.56	0.261	46.056	1.097	55.042	3.610	0.024
Gen (G)	23	4.130	6.260*	5.160	64.772**	901.09**	2.258**	42.309	3.128	8.922*	11.880	0.036*
Error	46	2.672	3.173	3.442	10.740	140.12	0.405	65.853	3.575	3.969	10.743	0.013
Total	71											
HS												
Rep	2	36.264	2.514	1.931	192.822	50672.40	.638	146.431	11.847	4.597	0.027	0.109
Gen (G)	23	9.759	2.898	9.012**	46.666	5261.3*	0.988**	37.766	3.666	12.280*	7.152	0.022
Error	46	10.974	4.760	2.554	46.513	140.12	0.267	78.416	3.746	7.061	7.659	0.024
Total	71											

HS: Heat Stressed, NS: Non-stressed, Rep: Replication, Gen: Genotype. *, **, significant at 0.05 and 0.01 level of probability

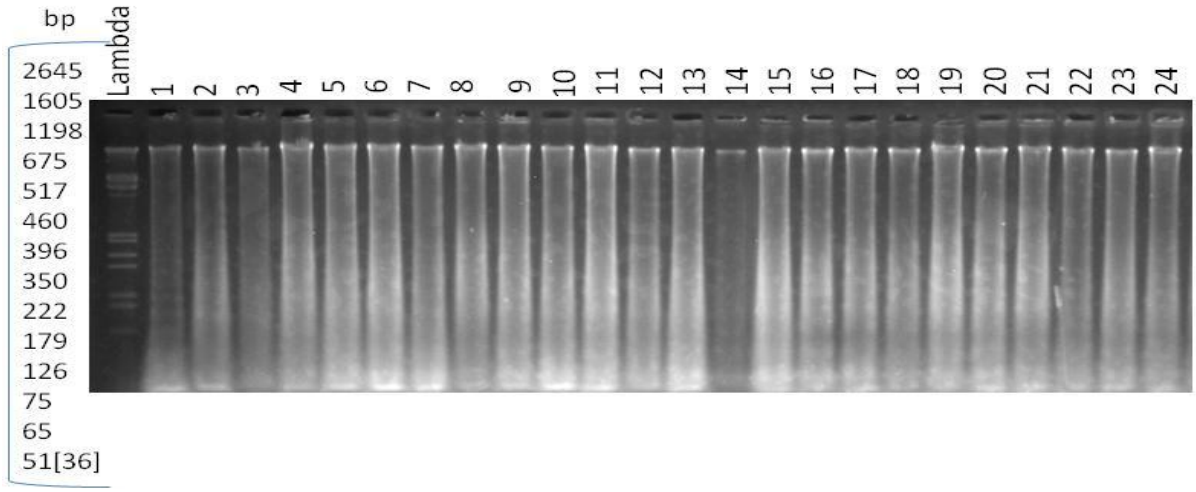

Plate 1: Quality test of 24 bread wheat accessions

Table 3: Major Allele Frequency, Number of Alleles, Genetic Diversity and PIC at Functional and Random DNA Markers Linked to Agronomic Traits, and Biotic Stresses Resistance in 24 Bread Wheat Lines.

Marker	Chromosome Position	Major Allele Frequency	Number of Obsevation	Number of Alleles	Genetic Diversity	PIC
Xgwm111	7D	0.5238	21.0000	4.0000	0.6304	0.5736
Xgwm140	1B	0.3043	23.0000	7.0000	0.7902	0.7604
Xgwm577	7B	0.2273	22.0000	9.0000	0.8471	0.8296
Xwmc44	1B	0.9583	24.0000	2.0000	0.0799	0.0767
Xgwm533	3B	0.7917	24.0000	3.0000	0.3438	0.3067
Xwmc89	4A	0.5833	24.0000	2.0000	0.4861	0.3680
iag 95	1B/1R	0.5000	24.0000	2.0000	0.5000	0.3750
PPd-D1	2D	0.9167	24.0000	2.0000	0.1528	0.1411
Dreb-B1	3BL	1.0000	24.0000	1.0000	0.0000	0.0000
Vrn-A1	5AL	0.7917	24.0000	3.0000	0.3507	0.3222
Rht-B1b	4B	0.9167	24.0000	2.0000	0.1528	0.1411
Rht-D1b	4D	0.9583	24.0000	2.0000	0.0799	0.0767
Total				39.0000		
Mean		0.7060	23.5000	3.2500	0.3678	0.3309
SD (±)				2.3789	0.2865	0.2708

PIC: Polymorphic Information Content

Table 4: Important gene traits of interest for wheat Breeding based on Analysis of Functional and Random DNA markers linked to Agronomic Traits

Locus	Type of Marker	Interesting allele designation/ size bp	Allele frequency in (%)
Xwmc89	Linked	120	58
iag95 (1BL/1RS)	Closely linked	1.1	50
Ppd-D1	Functional	*Ppd-D1 b* (414)	92
Dreb B1	Functional	717	100
VRN1AF/VRN1-INT1R-Vrn-A1/ Vrn-A1	Functional	*Vrn-A1a* (965 and 876) *Vrn-A1c* (484)	13 87
Xwmc44	Linked	242	25
Xgwm533-St b2	Linked	120	4
Xgwm111	Linked	220	17
Xgwm140	Linked	242	25
Xgwm577	Linked	150	21
Rht-B1 (Rht1)	Functional	*Rht-B1b* (237)	92
Rht-D1 (Rht2)	Functional	*Rht-D1b* (254)	4

Table 5: Shared allele genetic distance of 24 bread wheat accessions using 12 functional and linked markers

ACC	4401	4410	4411	4412	4413	4414	4415	4416	4417	4418	4419	4402	4420	4421	4422	4423	4424	4403	4404	4405	4406	4407	4408	4409
4401	0.000																							
4410	0.333	0.000																						
4411	0.333	0.333	0.000																					
4412	0.500	0.667	0.667	0.000																				
4413	0.417	0.417	0.250	0.750	0.000																			
4414	0.500	0.500	0.417	0.750	0.333	0.000																		
4415	0.167	0.167	0.333	0.500	0.333	0.417	0.000																	
4416	0.333	0.417	0.333	0.667	0.333	0.500	0.250	0.000																
4417	0.333	0.417	0.250	0.667	0.333	0.333	0.333	0.333	0.000															
4418	0.333	0.417	0.167	0.667	0.333	0.333	0.333	0.333	0.083	0.000														
4419	0.364	0.364	0.182	0.727	0.182	0.273	0.364	0.364	0.182	0.273	0.000													
4402	0.083	0.333	0.333	0.500	0.417	0.500	0.167	0.333	0.333	0.333	0.364	0.000												
4420	0.333	0.500	0.250	0.750	0.167	0.250	0.417	0.417	0.250	0.250	0.182	0.333	0.000											
4421	0.364	0.545	0.364	0.545	0.273	0.364	0.364	0.364	0.364	0.364	0.200	0.364	0.273	0.000										
4422	0.200	0.400	0.400	0.700	0.300	0.400	0.300	0.400	0.400	0.400	0.300	0.200	0.200	0.333	0.000									
4423	0.273	0.364	0.364	0.636	0.273	0.273	0.273	0.455	0.364	0.364	0.200	0.182	0.273	0.300	0.200	0.000								
4424	0.364	0.455	0.455	0.545	0.364	0.455	0.364	0.545	0.364	0.364	0.300	0.364	0.364	0.400	0.300	0.273	0.000							
4403	0.083	0.333	0.333	0.500	0.417	0.500	0.167	0.333	0.250	0.250	0.364	0.083	0.333	0.364	0.200	0.273	0.273	0.000						
4404	0.250	0.417	0.250	0.583	0.333	0.333	0.250	0.167	0.250	0.250	0.273	0.250	0.333	0.273	0.400	0.273	0.455	0.250	0.000					
4405	0.417	0.500	0.583	0.667	0.500	0.250	0.333	0.417	0.583	0.583	0.455	0.417	0.500	0.455	0.300	0.273	0.455	0.417	0.417	0.000				
4406	0.417	0.500	0.417	0.750	0.333	0.250	0.333	0.250	0.417	0.417	0.273	0.417	0.333	0.273	0.300	0.273	0.455	0.417	0.250	0.167	0.000			
4407	0.333	0.500	0.333	0.667	0.417	0.417	0.333	0.250	0.250	0.250	0.364	0.333	0.417	0.364	0.300	0.364	0.455	0.250	0.167	0.417	0.250	0.000		
4408	0.417	0.500	0.333	0.750	0.333	0.250	0.500	0.500	0.417	0.417	0.182	0.417	0.250	0.364	0.300	0.364	0.455	0.417	0.417	0.417	0.417	0.500	0.000	
4409	0.417	0.500	0.333	0.750	0.333	0.250	0.500	0.500	0.417	0.417	0.182	0.417	0.250	0.364	0.300	0.364	0.455	0.417	0.417	0.417	0.417	0.500	0.000	0.000

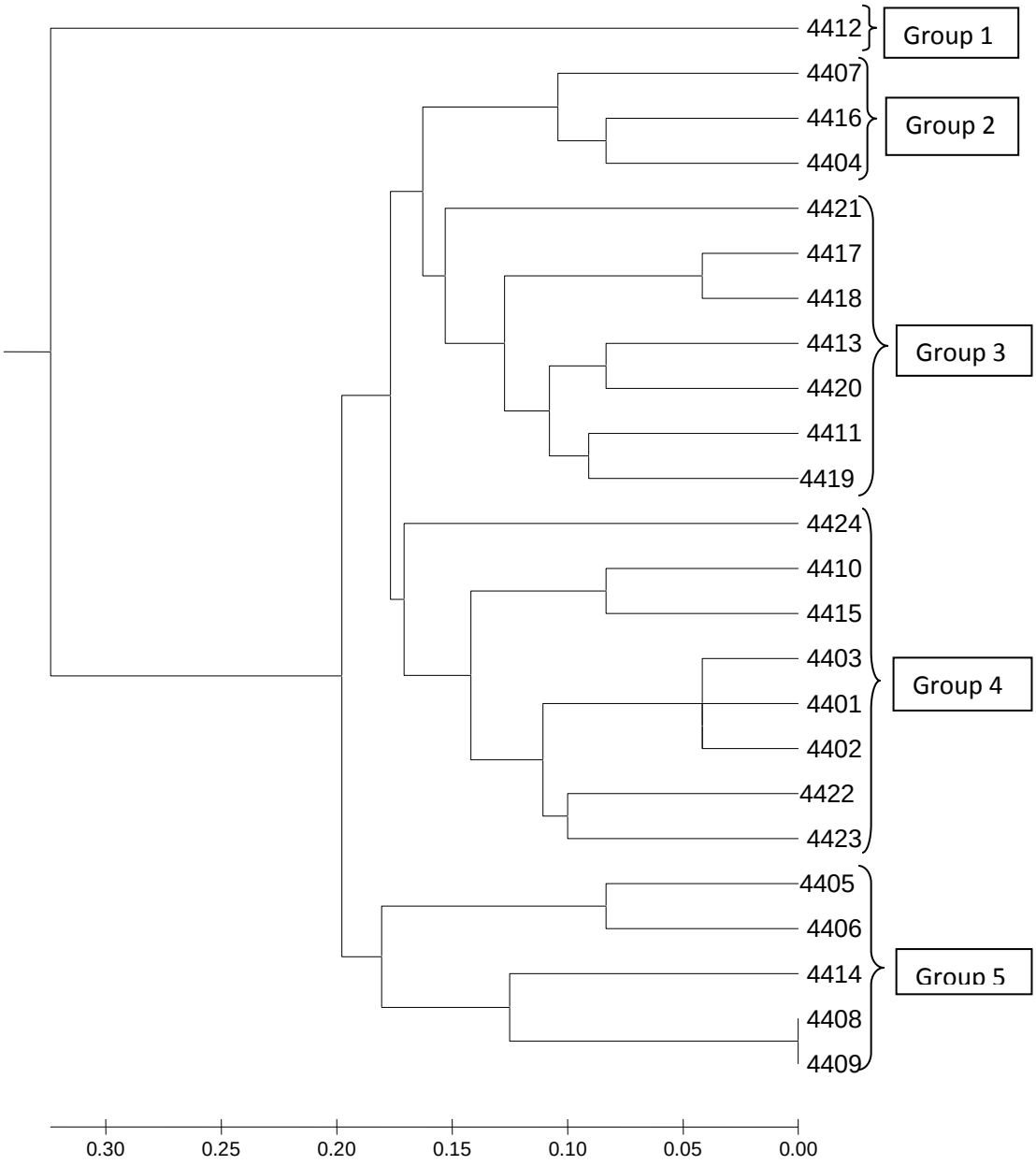

Fig. 1: Neighbor-Joining Dendrogram showing relationships among the 24 bread wheat accessions as revealed by the method based on shared allele genetic distance

Genetic diversity analysis

Plate 1 shows the result of quality test by the use of Agarose Gel Electrophosis; Table 3 present total numbers of detected alleles were 39; mean number of alleles were 3.25. Number of alleles observed and genetic diversity index differed among the loci tested. Number of alleles range from 1- (Dreb-B1) to 9- (Xgwm577). Similarly, genetic diversity index also differed greatly among the loci from 0.0000 in case of Dreb-B1 to 0.8471 in case of Xgwm577. The PIC value was also differed from 0.0000 (Dreb-B1) to 0.8296 (Xgwm577).

Markers based trait analysis

Table 4 shows allele frequencies random DNA marker allele at *Xgwm140* and *Xwmc4* were 25 % in each gene. Similarly, marker alleles *Xgwm577* and *Xgwm533* at 150 bp and 120bp have allele frequencies of 21% and 4%, respectively. Allele frequency of 1*BL.1RS* translocation was 50 % and 58% of allaele frequency showed presence of 120 bp size allele of *Xwmc89*. Functional marker alleles of *Dreb-B1* showed alleles frequency in all accessions. Linked marker allele *Xgwm111* showed 17% allele frequency at 220-bp. For the other agronomic traits, such as, dwarfing genes *Rht1* in Plate 5 and *Rht2*, the allele frequencies were 92% and 4%, respectively. 92% of allaele frequency at Ppd-D1 locus. While, VrnA1a and VrnA1c primer pair amplified at 965 and 876 bp and 484 bp fragments showed allele frequencies of 13 % and 87%, respectively.

Shared Alleles Genetic Distance

Table 5, the lowest genetic distance (0.083) were recorded between accessions 4402 and 4401; accessions 4403 and 4401; accessions 4403 and 4402; and accessions 4418 and 4417, indicating that these pair of accessions are closely related to each other. The highest genetic distance (0.750)

were observed between accessions 4409 and 4412, accessions 4413 and 4412, accessions 4414 and 4412, accessions 4420 and 4412, accessions 4406 and 4412, and accessions 4408 and 4412.

Figure 1 shows the dendrogram showing relationships between the 24 bread wheat accessions as revealed by the Neighbor-Joining method based on shared allele genetic distance were grouped into 5 at a genetic distance level of 0.15; accessions 4407, 4416, and 4414 grouped together and formed a single cluster. Accessions 4421, 4417, 4418, 4413, 4420, 4411, and 4419 formed a separated cluster. Other accessions are: 4424, 4410, 4415, 4403, 4401, 4402, 4422, 4423, and accessions 4405, 4406, 4414, 4408, and 4409 were embedded into group 4 and group 5, respectively. Moreover, accession 4412 was embedded in a single cluster.

Molecular marker analysis

The total numbers of allele detected at 12 loci were 39 alleles in all cultivars (mean 3.25 alleles). The PIC value was 0.33 for all cultivars. Similar studies conducted by Vanzetti *et al.* (2013) for 102 Argentinean bread wheat cultivars reported an average number of alleles and PIC values of 3.26 and 0.458, respectively. In India, Malik *et al.* (2013) characterized 48 elite Indian wheat genotypes reported to have 2.42 alleles per locus and 0.4596 PIC value. The functional markers and the random DNA markers linked to the target traits shown to be ideal for marker assisted selection in wheat breeding, such as *Xgwm144* and *Xwmc44* which are associated with yellow and leaf rust genes. Those of *Xgwm577*and *Xgwm533* were linked to *Stb2* and *Stb8*, 1BL/1RS translocation, growth photoperiod sensitivity (Ppd-D1). Plant height (*Rht-B1, Rht-D1*), *Xwmc89*, and *Dreb-B1* which are closely associated with drought and heat tolerant genes.*Xgwm111* which is closely linked to heat tolerant gene and *VrnA1a* and *VrnA1c* linked to QTL for flowering time.The use of gene specific markers permitted to know the genetic structure of modern wheat cultivars. The functional alleles of some of these traits were related to the respective phenotypes of the cultivars, previously described by the breeders; all genotypes known for their resistance to heat and drought carrying *Ppd-D1* Yang *et al.* (2009*), Dreb-B1*, *Rht-B1* Ellis *et al.* (2002) and *Vrn-A1* gene Yan *et al.* (2006), were clearly amplified at the alleles of the marker tightly linked to abiotic stresses Singh *et al.* (1998). In addition, Accession 4401 and 4409 showed the presence of dwarfing gene allele *Rht-D1b* Ellis *et al*. (2002), which is also known for its large adaptation, high yield and tolerance to drought (Jlibene and Nsarellah, 2011). However, these cultivars need to be further improved by incorporating the heat tolerance resistance gene, which a major problem in the wheat growing regions of Nigeria. The linked random DNA analysis also revealed the possibilities of having the stem rust gene (*Sr2*) linked to *Xgwm533* (maswheatin) Accessions 4409 and 4424 which needs to be further confirmed based on the phenoty piccharacterization. These two cultivars with stem rust resistance genes could be valuable parents in wheat breeding program due the additive resistance effect resulted from combined stem rust genes. Furthermore, the analysis in this study also revealed that the cultivar 4401, 4402, 4404, 4406, and 4416 also carried Septoriatritici blotch resistance allele (*Stb8*) linked to *Xgwm577* and Accessions 4401, 4408, 4409, and 4412 carried *Stb8* locus allele linked to *Xgwm111* associated with heat tolerance gene Adhikari *et al.* (2004). Therefore, Accession 4401 is very valuable cultivar for use as donor in molecular breeding program. The cultivars 4402-4407, 4409, 4411, and 4413-4416 revealed the presence of iag95 marker specific for 1BL.1RS translocation Mago *et al.* (2002). Accessions 4401-4403, 4406–4409, 4411, 4414-4416, 4419, and 4420 showed presence of grain yield under drought stress allele Dharwar Dry (drought tolerant)/Sitta: SSR locus *Xwmc89-4AL* was the marker most closely associated with QTL for grain yield; grains fill rate, spike density, grains/m2, biomass and drought susceptibility index Somers *et al.* (2004). Similarly, the molecular analysis also revealed that Accessions 4403, 4405, 4407, 4409, 4411, 4418 and Accessions 4402, 4404, 4406, 4408, and 4413 carried the presence of leaf rust alleles (*Lr46*) which are linked to the DNA markers *Xwmc44* and *Xgwm140* (Dubcovsky *et al.*, 1998). The Neighbor-Joining dendrogram and results revealed a clear differentiation between groups indicating that the genotypes used in this study, were divergent and can be used to improve heat stress resistance, quality and also genetic diversity.

Conclusion

In conclusion, this study had extensively investigated and established vital molecular and phenotyphic information for identifying promising genotypes with good breeding values of important agronomic characters for developing high yielding, and more importantly heat tolerance on bread wheat. Information of genetic diversity, identification of specific alleles, genes or loci and assessment of the genetic relationships among these cultivars can provide relevant guidelines in selecting parents and for designing new breeding strategies for wheat cultivar improvement, especially, against heat and drought tolerance which are considered as most destructive abiotic stresses in wheat production. To maintain growth and productivity, crops must adapt to stress conditions and exercise specific tolerance mechanisms. In this study genotypes 4404, 4408, 4410, 4411, 4413, 4414, and 4420 were identified as top yielders as such could be explored for resistance lines against heat stress.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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