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Genetic Advance in Heat Tolerant Bread Wheat (*Triticum Aestivum* L.) Lines Using Conventional and Molecular Maker Techniques

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Abstract

Field trials were conducted at Lake Chad Research Institute Wheat 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 from bread wheat, the Quantitative Trait Loci (QTL) associated with yield and selected agronomic traits, and to explore the potential of marker-assisted selection (MAS) in improving wheat heat tolerance. One hundred and twenty-six (126) wheat lines were screened in breeding nursery and twenty-four (24) lines were selected based on high yielding during the 2014/2015 dry season. 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 selected 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 30cm row spacing apart. The analysis of variance for individual environment was computed using the General Linear Model (GLM) SAS version 9.2. Wizard Genomic DNA Purification Kit was used for DNA extraction. 24 lines were selected for DNA

extraction. Total genomic DNA was extracted by Cetyltrimethylammonium Bromide (CTAB) method. Total of 5 functional markers and 7 linked Random DNA Markers to the traits of interest were used for genotyping the bread wheat cultivars. The means square from analysis of variance for the individual environment for growth and yield characters under normal and heat stressed condition indicated that highly significant differences were observed between genotypes. The results 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 genetic distance was recorded between accessions; 4402 and 4401, 4403 and 4401, 4403 and 4402, 4418 and 4417 at genetic distance level of 0.083, indicating that these accessions are closely related to each other. Highest genetic distance was 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 (0.750). Cluster analysis had grouped the accessions into 5 groups at a genetic distance level of 0.15. In conclusion, this study had extensively investigated and established vital molecular and phenotyphic information for identifying promising genotypes with good breeding values. In this study genotypes 4404, 4408, 4410, 4411, 4413, 4414, and 4420 was identified as top yielder as such could be explored for resistance lines against heat stress.

Introduction

Wheat (*Triticum aestivum* L. em Thell.) is the first important and strategic cereal crop for the majority of world's populations (FAO, 2010). The grass family Poaceae includes major crop plants such as wheat, barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), rye (*Secale cereale* 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 insurgency activities are growing stronger in many of these areas and local wheat production has declined during these years. Unfavorable climatic conditions requiring expensive irrigation also make it not competitive to grow wheat within this otherwise wheat suitable growing belt (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 25^{o}C (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.,* 1999; 2000; Dias, and Lidon, 2009).

The average global temperature is reported to be increasing at a rate of 0.18 °C every decade (Hansen *et al.,* 2012). In Nigeria (Guinea Savanna), a substantial wheat area is heat stressed due to high temperature and changes in the climate pattern, development of heat tolerant varieties and generation of improved pre breeding material seems to be the main focus of any breeding program in future (Ortiz *et al.,* 2007). However, due to complex nature of heat stress tolerance, population growth, the advanced techniques of molecular breeding and genetic engineering including MAS in combination with

conventional breeding approaches can play a vital role in designing new wheat cultivars with enhanced heat tolerance.

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 difficult to select under field conditions and that are controlled by multiple genes.

The study was focused on genetic advance in heat tolerant bread wheat lines using conventional and molecular marker techniques**.** The Objectives of the Study were to; identify and evaluate heat tolerant lines from bread wheat, identify Quantitative Trait Loci (QTL) associated with yield and selected agronomic traits, and to explore the potential of marker-assisted selection (MAS) for improving heat tolerance in wheat.

MATERIALS AND METHODS

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 600m above sea level. One hundred and twenty-six (126) wheat lines screened in breeding nursery and 24 lines (Table 1) were selected based on high yield during the 2014 dry season. Normal sowing (15th November) sowing was non-stressed; heat stress was imposed through staggered (6th January) resulted in terminal heat stress. The selected 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 N was given in three split doses at planting, and at four and eight weeks after planting. Data were recorded as described by Abdullahi, (2013). The form of analysis of variance for individual environment was computed using the General Linear Model (GLM) in SAS version 9.2 (SAS Institute Inc., 2009, SA).

DNA Extraction and Marker Genotyping

Wizard Genomic DNA Purification Kit was used for DNA extraction. Fresh young leaves (30 mg) were collected from individual cultivars were used for DNA extraction and marker analysis, total genomic DNA was extracted by CTAB method which was modified by Udupa *et al.* (1999).

Agarose Gel Electrophoresis

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). Electrophoresis was first run at 60 V 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 $MgCl_2$), 200µM of each dNTPs, 10 pmole of each primer, 0.5 U of Taq DNA polymerase (Promega) and approximately 50 ng of genomic DNA. The amplification reaction was performed in the Eppendorf Master cycler with an initial denaturation for 5 minutes at 94°C, followed by 35 cycles of each cycle with 30 seconds denaturation at 94°C, 30 seconds annealing at X°C, (depending on the primer pair), 45 seconds extension at 72°C. Final extension was carried out at 72°C for 5 minutes followed by cooling at 4°C for infinite period. Amplified products were separated on 1.2 or 1.5 % (w/v) agarose gels for functional markers. Linked markers were run in 6 % (w/v) native polyacrylamide gels, 6% (w/v) denaturing polyacrylamide gels. The amplified bands were detected by silver staining. Size of each band was estimated simultaneously by means of a 100-bp DNA Ladder.

PowerMarker software version 3.25 (Liu and Muse 2005) was used to estimate the number of alleles, genetic diversity and PIC (Botstein *et al.* 1980) of each locus. 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 informativeness of the SSR markers, the polymorphic information content (PIC) for each microsatellite were estimated. PIC values were estimated according to Anderson *et al.* (1993) as:

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

Alleles amplified by microsatellite primers for each cultivar were scored and genetic diversity (*H*) was calculated (Nei, 1987):

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

RESULTS AND DISCUSSION

Table 2 shows mean squares from the Analysis of Variance for the phenotypic characters (growth and yield) under normal and heat stressed, evaluated in 2015/2016 cropping season. The results indicated that highly significant differences were recorded between genotypes for plant height, number of tillers, and spike length at 1% probability level. Furthermore, significant differences 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 highly significant differences ($P<0.01$) between genotypes for days to 50% flowering and spike length. While, significant differences ($P<0.05$) were also observed between the genotypes for number of tillers and days to 50% maturity. Significant differences were observed among the genotypes for most of the growth and yield characters. This indicates the presence of appreciable amounts of genetic variability in the accessions studied. This further implies that the genotypes derived from this study will likely respond for selection, which is in line with the report of Falconer (1989), that any amount of improvement obtained by selection among a number of genotypes is dependent on the amount of variability between genotypes and the intensity of selection.

The major allele frequency, number of alleles, genetic diversity and polymorphic information content (PIC) at the functional and random DNA markers linked to agronomic traits, and biotic stresses resistance in 24 bread wheat lines are presented in Table 3. The total numbers of detected alleles were 39; mean number of alleles were 3.25. Similar studies have been conducted by Vanzetti *et al.* (2013) for 102 Argentinean bread wheat cultivars and 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. Number of alleles observed and genetic diversity index varied among the loci tested. Number of alleles range from 1- (Dreb-B1) to 9- (Xgwm577).

Similarly, genetic diversity index also varied greatly among the loci from 0.0000 in case of Dreb-B1 to 0.8471 in case of Xgwm577. The PIC value was also varied from 0.0000 (Dreb-B1) to 0.8296 (Xgwm577). 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 (CIMMYT, 2001). Lombardi *et al.* (2014) reported that selection of divergent parental genotypes for breeding should be made actively on the basis of systematic assessment of genetic distance between genotypes, rather than passively based on geographical distance.

Table 4 shows important gene traits of interest for wheat breeding based on analysis of the functional and random DNA markers linked to agronomic traits. The allele frequencies random DNA marker allele at *Xgwm140* and *Xwmc44* were 25 % in each gene. Similarly, marker alleles *Xgwm577* and *Xgwm533* at 150bp and 120bp have allele frequencies of 21% and 4%, respectively. Allele frequency of 1*BL.1RS* translocation was 50 % and 58% of allele 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* and *Rht2*, the allele frequencies were 92 % and 4 %, respectively. 92 % of allele 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. The functional markers and the random DNA markers linked to the target traits such as the *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*, which is closely associated with QTL for drought tolerance, *Dreb-B1* also 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 shown to be ideal for marker assisted selection in wheat breeding.

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* Bo *et al. (*2008), *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 (*http://maswheat.ucdavis.edu/protocols/index.htm*). 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* (http://maswheat.ucdavis.edu/protocols/Lr46/index.htm) in Accessions 4409 and 4424, which needs to be further confirmed based on phenotypic char acterization. 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 Lillemo *et al.* (2011). Furthermore, the analysis in this study also revealed that the cultivar 4401, 4402, 4404, 4406, and 4416 also carried Septoria tritici blotch resistance allele (*Stb8*) linked to *Xgwm577* Röder *et al.* (1998) and Accessions 4401, 4408, 4409, and 4412 carried *Stb8* locus allele linked to *Xgwm111* associated with heat tolerance gene (http://maswheat.ucdavis.edu/protocols/Stb4/index.htm). 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* (http://maswheat.ucdavis.edu/protocols/Lr46/index.htm). 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.

The shared alleles genetic distance of 24 bread wheat accessions using functional and linked markers are presented in 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. Further more; accession 4412 was embaded in a single cluster.

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 AND RECOMMENDATION

In conclusion, this study had extensively investigated and established vital molecular and phenotypic 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. 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 resistance lines against heat stress.

REFERENCES

Abbas, M. (1988). First CIMMYT (Mexico)/NAFPP/AERLS/NSS Joint one-week intensive training course in *International Wheat Production.* Jan. 18th -23rd. 1-3.

Abdullahi, T. A. (2013). Lake Chad Research Institute dry season wheat research activities at

Kirenowa, Marte Local Government Area, Borno State. *Field data recording.* Unpublished

Anderson, J. A., Sorrells, M. E., and Tanksley, S. D. (1993). RFLP analysis of genomic

Regions associated with resistance to preharvest sprouting in wheat. *Crop Science*. 33: 453-459.

Bo, W., Ruilian J. Chengshe, W. Jibao, C. Xinguo, M. Xiaoping, C. and Jizeng, J. (2008).

Dreb1 genes in wheat (*Triticum aestivum* L.): development of functional markers and gene mapping based on SNPs. Springer Science+Business Media B.V. 2008.

Botstein, C. M., Carter, T. E. Jr., Bailey, M. A. Mian, M. A. R., Rufty, T.W., Ashley, D. A., Boerma, H.R., Arellano, C., Hussey, V., and Parrott, V. (1980). Aluminum tolerance associated with quantitative trait loci derived from soybean PI 416937 in hydroponics. *Crop Science* 40: 538-545.

Boyer, J.S. (1982). Plant productivity and environment. *Science,*218: 443-448

CIMMYT. (2001). World wheat Overview and Outlook. World wheat Overview
andOutlook. *http://www.cimmyt.org/Research/Economics/map/facts_trends/wheat00-01/pdf/wheato&o00-01_part2.pdf*.

Dias, A.S., and Lidon, F.C. (2009). Evaluation of grain filling rate and duration in bread and durum wheat, under heat stress after anthesis *Journal of Agronomy and Crop Science.* 195: 137-147.

Ellis, M.H., Spielmeyer, W., Gale, K.R., Rebetzke, G.J. and Richards, R.A. (2002). Perfect markers for the Rht-B1b and Rht-D1b dwarfing genes in

wheat. *Theory and Applied Genetics.* 105:1038–1042. doi:10.1007/s00122-002-1048-4.

Falconer, D. S., and Mackay, T. F. C. (1996). Introduction to quantitative genetics. 464. *Longman Group Ltd. Essex,* England.

F.A.O. (2010). Statistical Division, *http//faostat.Fao.org* Fu, D.L., Li, C., Blechl, A., Tranquili, G., Bonafede, M., Sanchez, A., Valarik, M., Yasuda,

S. and Dubcovsky, J. (2006). The wheat and barley vernization gene VRN3 is an orthologue of FT. *Proceeding of Natational Academy of Science* USA 103:19581-19586. DOI:10.1073/pnas.0607142103.

Georgieva, K. (1999). Some mechanisms of damage and acclimation of the photosynthetic appararatus due to high temperature. *Bulgerian Journal of Plant Physiology. 25*: 89-100.

Habash, D. Z., Kehel, Z. and Nachit, M. (2009). Genomic approaches for designing durum wheat ready for climate change with a focus on drought. *Journal of Experimental Botany* 60(10): 2805–2815

Hansen, J., Sato, M.K., and Ruedy, R. (2012). Perception of climate change. *Proceeding of National Academy of Science 109:14726–14727.*

Hassan, I.A. (2006). Effects of water stress and high temperature on gas exchange and chlorophyll fluorescence in *Triticum aestivum* L. *Photosynthetica,* 44: 312-315.

Hays, D.B., Do, J.H., Mason, R.E., Morgan, G., Finlayson, S.A. (2007). Heat stress induced ethylene production in developing wheat grains induces kernel abortion and increased maturation in a susceptible cultivar *Plant Science.* 172(6):1113-1123. http://maswheat.ucdavis.edu/protocols/*Lr46/Stb4/Sr2/index*.htm

Jin, L. and Chakraborty, R. (1993). Estimation of genetic distance and coefficient of gene diversity from single-probe multilocus DNA fingerprinting. Data *Molecular Biology Evolution* 11:120–127.

Jlibene, M. and Nsarellah, N. (2011). Wheat breeding in Morocco, a historical perspective. In: Angus WJ, Bonjean A, van Ginkel M (eds) *The world wheat book, vol 2.* A history of wheat breeding. Lavoisier Publishing, Paris, pp 425–442.

Lillemo, M., Singh, RP., William, M., Herrera-Foessel, SA., Huerta-Espino, J., Germa´n, S., Campos, P., Chaves, M., Madriaga, R., Xia, X., Liang, S.,

Liu, D., Li, Z. and Lagudah, E. (2011). Multiple rust resistance and gene additivity in wheat: lessons from multi-location case studies in the cultivars Parula and Saar. In: McIntosh RA (ed) Technical *Workshop, St. Paul, Minnesota, USA*, p 111–120.

Liu, K. and Muse, S.V. (2005). PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21:2128–2129. doi:10.1093/bioinformatics/bti282.

Macas, B., Gomes, C. and Dias, A. S. (1999). *Melhoramento*, 36: 27-45.

Macas, B., Gomes, M.C., Dias, A.S. and Coutinho, J. (2000). The tolerance of durum wheat to high temperatures during grain filling. In: Royo, C., Nachit, M. M., Di Fonzo, N. and Araus, J.L. eds. *Options Me´diterrane´ennes*. Durum Wheat Improvement.

Mago, R., Spielmeyer, W., Lawrence, G.J., Lagudah, E.S., Ellis, J.G. and Pryor, A. (2002). Identification and mapping of molecular markers linked to rust resistance genes located on chromosome 1RS of rye using wheat-rye translocation lines. *Theory and Applied Genetics* 104:1317–1324. doi:10.1007/s00122-002-0879-3

Malik, R., Tiwari, R., Arora, A., Kumar, P., Sheoran, S., Sharma, P., Singh, R., Tiwari, V. and Sharma, I. (2013). Genotypic characterization of elite Indian wheat genotypes using molecular markers and their pedigree analysis. *Australian Journal of Crop Science* 7:561–567

Mustapha, S. (1998). Wheat production in Nigeria: Past, Present and Future Prospect. In: Valencia, J. A., Salako, E. A., Ikwelle, M. C., Aminu-Kano, M., Abubakar, I. U., Miko, S., and Jaryum, J. Wheat production in Nigeria-Prospects and Constraints *Proceedings of the National Wheat Production Workshop.* February 17-18. 14-24.

Nei, M., 1987. *Molecular evolutionary genetics*. Columbia University Press, New York.

Olaoye, G., Bello, O. B., Ajani, A. K. and Ademuwagun, T. K. (2005). Changes In quantitative and qualitative characters in tne segregating F_1 population of sweet corn (*Zea mays* L.) crosses *Proceeding of the 30th Annual Conference of the Genetic Society of Nigeria* 5-6 September. University of Nigeria, Nsukka. Pp. 74-84.

Ortiz, R., Iwanaga, M., Reynolds, M.P., Wu, H., and Crouch, J.H. (2007). Overview on crop genetic engineering for drought-prone environments. *Journal of Agricultural Research 4.*

Röder, M. S., Korzun, V., Wendehake, K., Plaschke, J., Tixer, M. H., Leroy, P., and Ganal, M. W. (1998). A microsatellite map of wheat. *Genetics* 149.

SAS Institute Inc. (2009) SAS Version 9.2. Cary, NC: *SAS Institute Inc.*

Skovmand, B., Villareal, R., Van Ginkel, M., Rajaram, S. and Ortiz-Ferrara G. (1997). Semi dwarf bread wheat: names, parentages, pedigrees and origins. CIMMYT, Mexico *http://hdl.handle.net/10883/1372*. Accessed 17 Aug 2015.

Somers, D.J.; Isaac, P.; Edwards, K. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theory and Applied Genetics* 109: 1105-1114.

Udupa, S.M., Robertson, L.D., Weigand, F., Baum, M. and Kahl, G. (1999). Allelic variation at (TAA)n microsatellite loci in a world collection of chickpea (*Cicer arietinum* L.) germplasm. *Molecular Genetics Genome* 261:354–363. doi:10.1007/s004380050976

USDA. (2015). USDA, Washington, DC.*CropProduction.pdf (last accessed on April 8,2013).Available at:* http://www.usda.ov/oce/weather/pubs/Annual;http://www.worldwheatproduction.com

Vanzetti, L.S., Yerkovich, N., Chialvo, E., Lombardo, L., Vaschetto, L. and Helguera, M. (2013). Genetic structure of Argentinean hexaploid wheat germplasm. *Genetics of Molecular Biology* 36:391–399. doi:10.1590/S1415-47572013000300014.

Wahid, A., Gelani, S., Ashraf, M. and Foolad, M. (2007). Heat tolerance in plants: an overview. *Environmental and Experimental Botany* 61(3):199-223.

Wardlaw, I. and Wrigley, C. (1994). Heat tolerance in temperate cereals: an overview *Functional Plant Biology* 21(6):695-703.

Yan, L.L., Fu, D.L., Li, C., Blechl, A., Tranquili, G., Bonafede, M., Sanchez, A., Valarik, M., Yasuda, S. and Dubcovsky, J. (2006). The wheat and barley vernization gene VRN3 is an orthologue of FT. *Proceeding of*

Natational Academy of Science USA 103:19581-19586. DOI:10.1073/pnas.0607142103.

Yang, F.P., Zhang, X.K., Xia, X.C., Laurie, D.A., Yang, W.X. and He, Z.H. (2009). Distributionof the photoperiod insensitive Ppd-D1a allele in Chinese wheat cultivars. *Euphytica* 165:445–452. doi:10.1007/s10681-008-9745-y.

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/C	**MX110-**

	BRD CMSS06Y01026T-099TOPM-099Y-099ZTM-099Y-099M-18WGY-…	**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

Table 2: Mean Squares (MS) from the Analysis of Variance for Phenotypic Characters (Growth and Yield) under Normal and Heat Stressed Evaluated 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 SPIKELET COUNT	SPIKE DAY TO MATURITY	SPIKE LENGTH (CM)	SEED 1000 GRAIN WEIGHT(G)	PER SPIKE (KG/PLOT)	YIELD PER SPIKE
REP	2	6.889	7.347	6.500	5.732	1441.56 NS 55.042	0.261	3.610	46.056	0.024	1.097
GEN (G)	23	4.130	6.260*	5.160	64.772**	901.09** 8.922*	2.258**	11.880	42.309	0.036*	3.128

ERROR	46	2.672	3.173	3.442	10.740	140.12 3.969	0.405 10.743	65.853 0.013	3.575
TOTAL	71								
HS									
REP	2	36.264	2.514	1.931	192.822	50672.4 4.597	0.638 0.027	146.431 0.109	11.847
GEN (G)	23	9.759	2.898	9.012**	46.666	5261.3* 12.280*	0.988** 7.152	37.766 0.022	3.666
ERROR	46	10.974	4.760	2.554	46.513	140.12 7.061	0.267 7.659	78.416 0.024	3.746
TOTAL	71								

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

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 OBSERVATION	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 (IBL/IRS)	Closely linked	1.1	50
PPD-D1	Functional	*Ppd-D1 b* (414)	92
DREB B1	Functional	717	100
VRN1AF/VRN1-INTIR-VRN-A1/ VRN-A1-2	Functional	*Vrn-A1a* (965 and 876)	13
		Vrn-A1c (484)	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

ACC: Accessions

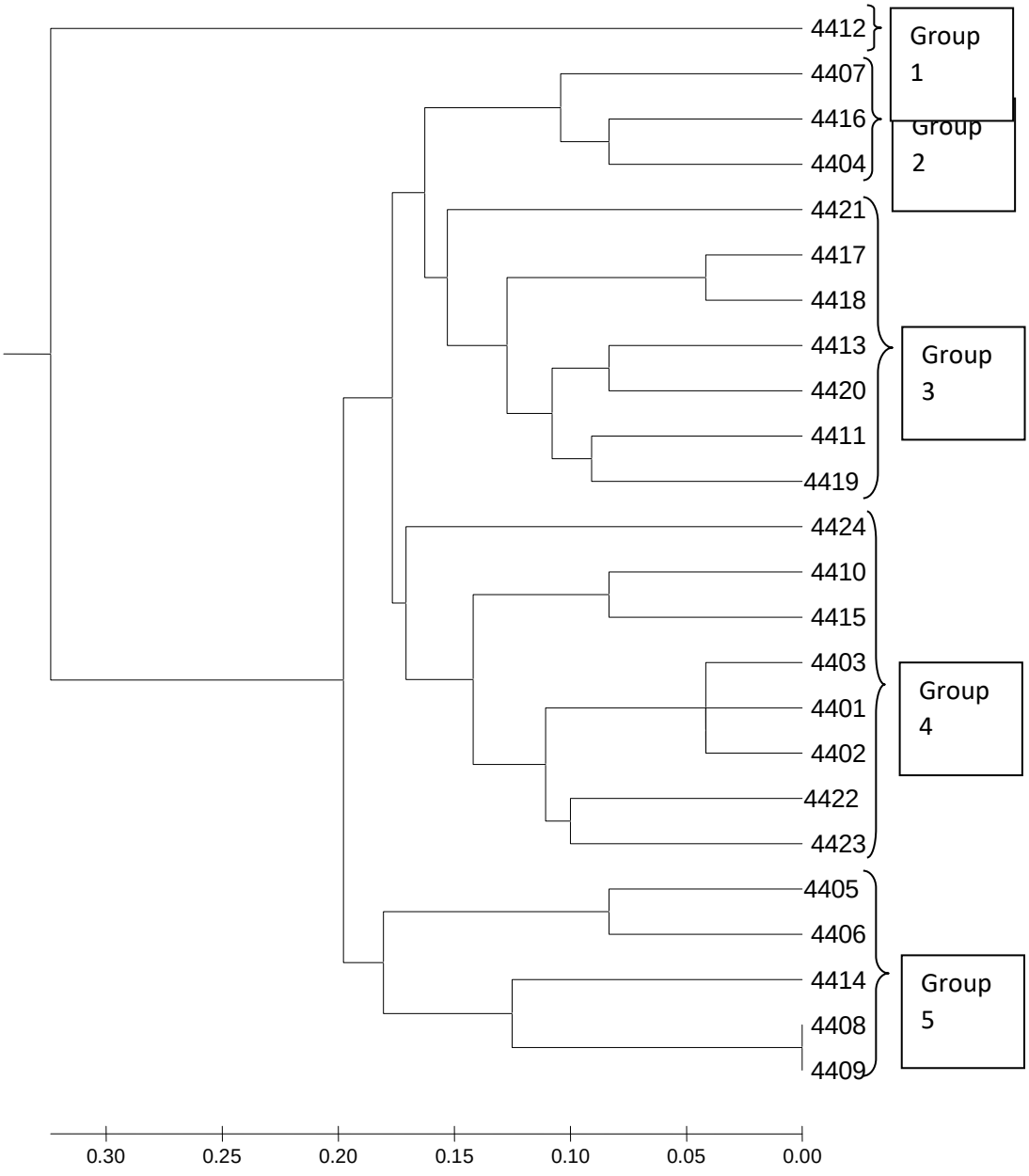

Figure 1: Neighbor-Joining Dendrogram showing relationships among the 24 bread wheat accessions as revealed by the method based on shared allele genetic distance