

Association mapping for heat tolerance in D-genome synthetic hexaploid wheats



**A PhD dissertation in partial fulfillment for the degree of doctor of philosophy in
Plant Sciences (Plant Biochemistry and Molecular Biology)**

by

Abdul Aziz

**Department of Plant Sciences,
Faculty of Biological Sciences,
Quaid-i-Azam University Islamabad**

2018

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Student/Author Signature: _____

Name: Abdul Aziz

Foreign Examiners

1. Dr. Zhonghu He Ph.D.

Research Professor,
Institute of Crop Science.
Chinese Academy of Agricultural Sciences (CAAS)
12 Zhongguancun South Street
Haidian District, Beijing 100081, PR China.
Phone: +86-10-82105691
Fax: +86-10-82108547
Email: hezhonghu02@caas.cn

2. Dr. Francis Ogonnaya, PhD

Senior Manager – Crop Genetics, Improving Crop Yield
Manager-Oil Seed and Pulses
Grain Research and Development Corporation (GRDC)
Level 4 East Building 4 National,
Circuit Barton ACT 2400 Australia.
Phone: +61-2-61664500
Fax: +61-2-61664599
Email: Francis.Ogonnaya@grdc.com.au

DECLARATION

This is Certify that the dissertation entitled ‘**Association mapping for heat tolerance in D-genome synthetic hexaploid wheats**’ submitted by **Abdul Aziz**, is accepted in its present form by the Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan as satisfying the dissertation requirements for the degree of **Doctor of Philosophy (PhD)** in Plant Sciences (**Biochemistry and Molecular Biology**).

Supervisor:

Dr. Tariq Mahmood

External Examiner:

Dr Jalal ud din
Principal Scientific Officer
Wheat Wide Program (WWP)
National Agricultural Research Center (NARC),
Islamabad

External Examiner:

Dr Shaukat Ali
Principal Scientific Officer
National Institute of Genetics and Biotechnology
(NIGAB)
National Agricultural Research Center (NARC),
Islamabad

Chairperson:

Dr. Tariq Mahmood
Chairman
Department of Plant Sciences
Quaid I Azam University Islamabad

Dated: 07 /02 /2018

*In dedication to
my loving, caring and supportive
parents,
family and to my Elder Brother their
love, encouragement and support I could
never have accomplished this work*

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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
AM	Association mapping
ANOVA	Analysis of variance
bp	Base pair
BWP	Bahawlpur
Chr	Chromosome
Cm	Centimeter
cM	Centimorgan
CV	Coefficient of variation
DArT	Diversity array technology
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide phosphates
EDTA	Ethylenediaminetetraacetic acid
FDR	False discovery rate
GLM	General linear model
GWAS	Genome wide association studies
GY	Grain yield
h^2	Broad sense heritability
HS	Heat stress
ISD	Islamabad
LD	Linkage disequilibrium
MAF	Minor allele frequency
MAS	Marker assisted selection
MLM	Mixed linear model
NOR	Normal treatment
PCA	Principal component analysis

PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RAPD	Randomly amplified polymorphic DNA
SND	Sindh
SNP	Single nucleotide polymorphism
SPAD	Soil and plant analyzer development
SSR	Simple sequence repeat
Taq	Thermus aquaticus
μL	Microlitre

Abstract

Adaptation of wheat (*Triticum aestivum* L.) to high temperature could be improved by introducing genes from wild relatives. We evaluated the responses of 200 D genome synthetic hexaploid wheats (*T. turgidum* × *Aegilops tauschii*; 2n=6x=42; AABB¹D¹) to high temperature to determine their potential for wheat improvement under field and controlled conditions. Under controlled conditions at grain filling stage, wheat plants were exposed to a brief heat stress (3 days, 37/27 °C) 10 days after anthesis and the plants evaluated for a number of morphological and physiological traits. In total, 114 synthetic hexaploid wheats and 6 bread wheat genotypes were evaluated for different heat responses during the grain filling stage. Considerable genetic variation was observed among wheat genotypes for various heat responses, particularly for single grain weight, chlorophyll retention, rate and duration of grain filling. Overall, the findings suggested that more than one adaptation process contributed to heat tolerance. Generally, genotypes with more stable grain weight under heat tended to have particular traits under stress, including the ability to maintain chlorophyll content and rate and duration of grain filling. Therefore, these traits may provide appropriate selection criteria for improving heat tolerance in wheat.

Field experiments were conducted in two different temperature scenarios (normal sowing time, “NOR”, and late sowing time to expose the plants to heat stress, “HS”), for two years at three different locations, to assess the effect of terminal high temperature on yield related traits. High temperature stress overall led to 39% reduction in grain yield and significant reductions of 28% in days to heading, 26% in plant height, 16% in grain number m⁻², and 18% in thousand kernel weight. The six most heat tolerant synthetic hexaploid wheats (SHWs) on the basis of higher grain yield across environments were AUS30284, AUS33384, AUS30288, AUS3029, AUS33409, and AUS30629. In ridge regression analysis, individual agronomic traits explained between 8.74 to 35.2% of variation in grain yield under HS treatments, with an average of 30.47%. In NOR treatments, individual agronomic traits explained between 8.85 and 45.49% of the variation in grain yield, with an average of 34.45% across all traits. Although, days to heading was negatively correlated with grain yield in heat stressed environment, but unlike adapted germplasm it did not explained significant variation in grain yield. Thousand grain weight explained more of the variation in grain yield in all environments followed by grain numbers m⁻².

Biochemical and physiological traits under terminal heat stress we evaluated were evaluated under field conditions using 13 Synthetic hexaploid wheats. High temperature stress overall led to 24.26% reduction in chlorophyll-a, 28.16% in chlorophyll-b, 22.16% in total chlorophyll, and 31.73% membrane stability index leakage was observed. While an increase of 29.83% soluble sugar and 41.78% proline contents in heat stress compared to normal was observed. Grain yield was positively correlated with the thousand-kernel weight ($r= 0.67$) in normal and heat stress environment and Chlorophyll a, Chlorophyll b and total chlorophyll have significant positive correlation with membrane stability index ($r= 0.85, 0.63$ and 0.60) in both normal and heat environments, respectively.

Association mapping (AM) was used to find out marker-trait associations (MTA) in SHW for heat tolerance using DArT markers. Data from all field experiments was used to find significant MTAs. LD and population structure discovered a high amount of genetic diversity present within SHWs. Five key sub-populations were identified using population structure within this panel of association mapping. In total, 17 MTAs for grain yield related traits in heat stress and 14 MTAs in normal environments were identified on chromosome 1A, 3B, 3D, 5B, 6B and 7B. From these 31 MTAs, 14 MTAs were common in both heat and normal conditions. These MTAs can be candidates for cloning genes linked to heat tolerance studies.

In conclusion synthetics hexaploid showed significant variation for grain yield and physiological traits and highly tolerant synthetic hexaploids were identified that can be used further to develop high yielding varieties adapted under high temperature stress.

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Abdul Aziz

Date: 7th February, 2018

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Student/Author Signature: _____

Name: Abdul Aziz

Foreign Examiners

1. Dr. Zhonghu He Ph.D.

Research Professor,

Institute of Crop Science.

Chinese Academy of Agricultural Sciences (CAAS)

12 Zhongguancun South Street

Haidian District, Beijing 100081, PR China.

Phone: +86-10-82105691

Fax: +86-10-82108547

Email: hezhonghu02@caas.cn

2. Dr. Francis Ogonnaya, PhD

Senior Manager - Crop Genetics, Improving Crop Yield

Manager-Oil Seed and Pulses

Grain Research and Development Corporation (GRDC)

Level 4 East Building 4 National,

Circuit Barton ACT 2400 Australia.

Phone: +61-2-61664500

Fax: +61-2-61664599

Email: Francis.Ogonnaya@grdc.com.au

?

DECLARATION

This is Certify that the dissertation entitled 'Association mapping for heat tolerance in D-genome synthetic hexaploid wheats" submitted by Abdul Aziz, is accepted in its present form by the Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan as satisfying the dissertation requirements for the degree of Doctor of Philosophy (PhD) in Plant Sciences (Biochemistry and Molecular Biology).

Supervisor: _____

Dr. Tariq Mahmood

External Examiner: _____

Dr Jalal ud din

Principal Scientific Officer

Wheat Wide Program (WWP)

National Agricultural Research Center (NARC), Islamabad

External Examiner: _____

Dr Shaukat Ali

Principal Scientific Officer

National Institute of Genetics and Biotechnology (NIGAB)

National Agricultural Research Center (NARC), Islamabad

Chairperson: _____

Dr. Tariq Mahmood

Chairman

Department of Plant Sciences

Quaid I Azam University Islamabad

Dated: 07 /02 /2018

In dedication to
my loving, caring and supportive parents,
family and to my Elder Brother their
love, encouragement and support I could
never have accomplished this work

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LIST OF ABBREVIATIONS

AFLP Amplified fragment length polymorphism

AM Association mapping

ANOVA Analysis of variance

bp Base pair

BWP Bahawalpur

Chr Chromosome

Cm Centimeter

cM Centimorgan

CV Coefficient of variation

DArT Diversity array technology

DMSO Dimethylsulphoxide

DNA Deoxyribonucleic acid

dNTPs Deoxynucleotide phosphates

EDTA Ethylenediaminetetraacetic acid

FDR False discovery rate

GLM General linear model

GWAS Genome wide association studies

GY Grain yield

h² Broad sense heritability

HS Heat stress

ISD Islamabad

LD Linkage disequilibrium

MAF Minor allele frequency

MAS Marker assisted selection

MLM Mixed linear model

NOR Normal treatment

PCA Principal component analysis

PCR Polymerase chain reaction

QTL Quantitative trait loci

RAPD Randomly amplified polymorphic DNA

SND Sindh

SNP Single nucleotide polymorphism

SPAD Soil and plant analyzer development

SSR Simple sequence repeat

Taq Thermus aquaticus

µL Microlitre

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Abstract

Adaptation of wheat (*Triticum aestivum* L.) to high temperature could be improved by introducing genes from wild relatives. We evaluated the responses of 200 D genome synthetic hexaploid wheats (*T. turgidum* × *Aegilops tauschii*; $2n=6x=42$; AABBDDtDt) to high temperature to determine their potential for wheat improvement under field and controlled conditions. Under controlled conditions at grain filling stage, wheat plants were exposed to a brief heat stress (3 days, 37/27 °C) 10 days after anthesis and the plants evaluated for a number of morphological and physiological traits. In total, 114 synthetic hexaploid wheats and 6 bread wheat genotypes were evaluated for different heat responses during the grain filling stage. Considerable genetic variation was observed among wheat genotypes for various heat responses, particularly for single grain weight, chlorophyll retention, rate and duration of grain filling. Overall, the findings suggested that more than one adaptation process contributed to heat tolerance. Generally, genotypes with more stable grain weight under heat tended to have particular traits under stress, including the ability to maintain chlorophyll content and rate and duration of grain filling. Therefore, these traits may provide appropriate selection criteria for improving heat tolerance in wheat.

Field experiments were conducted in two different temperature scenarios (normal sowing time, "NOR", and late sowing time to expose the plants to heat stress, "HS"), for two years at three different locations, to assess the effect of terminal high temperature on yield related traits. High temperature stress overall led to 39% reduction in grain yield and significant reductions of 28% in days to heading, 26% in plant height, 16% in grain number m⁻², and 18% in thousand kernel weight. The six most heat tolerant synthetic hexaploid wheats (SHWs) on the basis of higher grain yield across environments were AUS30284, AUS33384, AUS30288, AUS3029, AUS33409, and AUS30629. In ridge regression analysis, individual agronomic traits explained between 8.74 to 35.2% of variation in grain yield under HS treatments, with an average of 30.47%. In NOR treatments, individual agronomic traits explained between 8.85 and 45.49% of

the variation in grain yield, with an average of 34.45% across all traits. Although, days to heading was negatively correlated with grain yield in heat stressed environment, but unlike adapted germplasm it did not explained significant variation in grain yield. Thousand grain weight explained more of the variation in grain yield in all environments followed by grain numbers m⁻².

Biochemical and physiological traits under terminal heat stress we evaluated were evaluated under field conditions using 13 Synthetic hexaploid wheats. High temperature stress overall led to 24.26% reduction in chlorophyll-a, 28.16% in chlorophyll-b, 22.16% in total chlorophyll, and 31.73% membrane stability index leakage was observed. While an increase of 29.83% soluble sugar and 41.78% proline contents in heat stress compared to normal was observed. Grain yield was positively correlated with the thousand-kernel weight ($r= 0.67$) in normal and heat stress environment and Chlorophyll a, Chlorophyll b and total chlorophyll have significant positive correlation with membrane stability index ($r= 0.85, 0.63$ and 0.60) in both normal and heat environments, respectively.

Association mapping (AM) was used to find out marker-trait associations (MTA) in SHW for heat tolerance using DArT markers. Data from all field experiments was used to find significant MTAs. LD and population structure discovered a high amount of genetic diversity present within SHWs. Five key sub-populations were identified using population structure within this panel of association mapping. In total, 17 MTAs for grain yield related traits in heat stress and 14 MTAs in normal environments were identified on chromosome 1A, 3B, 3D, 5B, 6B and 7B. From these 31 MTAs, 14 MTAs were common in both heat and normal conditions. These MTAs can be candidates for cloning genes linked to heat tolerance studies.

In conclusion synthetics hexaploid showed significant variation for grain yield and physiological traits and highly tolerant synthetic hexaploids were identified that can be used further to develop high yielding varieties adapted under high temperature stress.