FUNCTIONAL GENOMIC STUDIES TO IDENTIFY LEAF RUST RESISTANCE RELATED GENES IN BREAD WHEAT

By

MOHAMMED MOSTAFA ELMAHDY ABD ALLAH

B.Sc. Agric. Sc. (Genetics), Ain Shams University, 2010

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ABSTRACT

Mohammed Mostafa El-mahdy Abd-Allah: Functional Genomic Studies to Identify Leaf Rust Resistance Related Genes in Bread Wheat. Unpublished M.Sc. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2017.

Wheat, one of three most important commercial crops around the world, is considered the first strategic food crop in Egypt. Wheat leaf rust caused by *Puccinia triticina Eriks*, reduces the yield and quality of the grain and yields. It cause yield loss between 20-50% if infection occurs very early. Differential display reverse transcription PCR (DDRT-PCR) was used to study the response of bread wheat to leaf rust infection at the molecular level. Three Egyption cultivars of bread wheat were used in this study; Shandwell 1, Gemmeiza 1 and Sids 1 representing resistant, moderately resistant and sensitive cultivars, respectively. Performance of non infected cultivars and after one and two weeks from infection was evaluated. Two hundred and forty eight differentially expressed fragments were observed as up-regulated, up- & down-regulated, down- & up-regulated and Down-regulated EST. Sixty four fragments were selected, extracted from the gel, reamplified and sequenced. BLAST program from National Center for Biotechnology Information (NCBI), USA were used to determine similar sequences of these fragments. Results of the database sequence alignment identified fragments with significant homology to genes and/or proteins with known function, other fragments with homology to unknown or hypothetical ESTs with unknown functions, and some fragments revealed no homology in the databases. These results implicated that several pathways are involved in the plant's response to leaf rust infection which still needs to be elucidated further.

Key words: Bread wheat, Biotic stresses, Leaf rust, *Puccinia triticinia*, Differential display reverse transcription (DDRT), PCR, cDNA, Gene expression and GenBank Database.

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LIST OF ABBREVIATIONS

Abbreviation	Full Name
Ap1	Arbitrary primer 1
Ap2	Arbitrary primer 2
Ap3	Arbitrary primer 3
Ap4	Arbitrary primer 4
Ap5	Arbitrary primer 5
Ap6	Arbitrary primer 6
Ap7	Arbitrary primer 7
Ap8	Arbitrary primer 8
AFLP	Amplified Fragment Length Polymorphism
ATP	Adenosine triphosphate
BLAST	Basic Local Alignment Search Tool
BLASTn	Nucleotide blast (Search a nucleotide database using a nucleotide query)
cDNA	Complementary DNA
CTAB	Cetyltrimethyl Ammonium Bromide
DD	Differential display
ddH2O	Double-distilled water
DDRT- PCR	Differential display reverse transcription-PCR
DNase	Deoxyribonuclease
dNTP	Deoxynucleotide
EDTA	Ethylenediaminetetraacetic acid
ESTs	Expressed Sequencing Tags
EtBr	Ethidium bromide stain

$MgCl_2$	Magnesium chloride
mRNA	messenger RNA
Na_2So_3	Sodium sulfite
NaCl	Sodium chloride
NaI	Sodium iodide
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain Reaction
Re-PCR	Reamplified-PCR
rRNA	Ribosomal RNA
rpm	Round per minute
SAM	S Adenosylmethionine
$T_{19}A$	Anchored Primer A
TAE	Tris-acetate-EDTA
TBE	Tris Borate EDTA

INTRODUCTION

Wheat is one of three most important commercial cereal crops around the world, It ranks first as the largest cultivated area which surpasses any other commercial crop. Wheat contains protein higher than the other major; cereals, maize or rice. It providing more than 20% of all calories consumed by the people worldwide. **FAO**'s latest forecast for 2015 global wheat production stands at 719 million tonnes **(FAO, 2015)**. In 2016, total Egyptian cereal harvest is 22.1 million tonnes, including nine million tonnes wheat production, this means that wheat represents 40.7 % of the total Egyptian cereal production **(FAO, 2016)**. Bread making is a basic staple food for both urban and rural areas. It is the main source of plant protein in the human nutrition.

World population is increasing at an alarming rate and is expected to reach about nine billions by the end of year 2050. On the other hand, food productivity is decreasing due to the effect of various abiotic and biotic stresses. Therefore minimizing these losses is a major area of concern for all nations to cope with the increasing food requirements **(Waraich *et al.*, 2012)**. Nature including plants always encounter many changes, that lead to various stress whether abiotic stress (temperature changes, drought and salinity) or biotic stresses (viruses, bacteria and fungus). On the other hand, researches and tecnology always develop new solutions. In order to find suitable solutions, different situations, reasons and conditions must be studied, here is the important role of the molecular biologists, because natural changes lead to changes on physiological responses of cells at the molecular level **(Harb 2010)**.

Globally important fungal diseases of wheat, caused by biotrophs (obligate parasites) include the three rusts (leaf rust *Puccinia triticinia*, stem rust *Puccinia graminis tritici* and yellow rust *Puccinia striiformis*), powdery mildew, and the bunts and smuts, whereas, those caused by hemibiotrophs (facultative parasites) include *Septoria tritici* leaf blotch, *Septoria nodorum* blotch, spot blotch, tan spot, and *Fusarium* head blight.