GENETIC STUDIES ON BREAD WHEAT RESISTANCE TO STEM RUST

BY

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B. Sc. Agric. Sc. (Biotechnology), Cairo University, 2003

M. Sc. Agric. Sc. (Genetics), Ain Shams University, 2009

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ABSTRACT

Ahmed Mohamed Mostafa Ramadan: Genetic Studies on Bread Wheat Resistance to Stem Rust. Unpublished Ph.D. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams . 6University, 201

Screening experiment was performed on twelve varieties and lines of bread wheat *(Ttriticum aestivum* L.*)* to select the most contrasting genotypes in stem rust susceptibility. The results of the experiment revealed that the resistant variety is (Misr1) and the two most stem rust susceptible lines are (Line37 and Line92) according to stem rust reaction. Crosses were carried out between the resistant parent (Misr1) with each of the two susceptible parents as well as between the two susceptible parents (Line37 and Line92) to obtain the F_1 kernels. Some of the F_1 kernels were sown in the field and self-pollinated to obtain the F_2 plants for each cross. These three selected parents, their F_1 and the most resistant and susceptible F_2 plant groups for the three crosses were evaluated for their response to stem rust resistance by recording some stem rust–related traits. However, the infection reduced the values of all yield related traits except spike length and number of spikelet's/spike traits. The three parents, their F_1 plants and some individual plants of the two contrasting F_2 plant groups as the offspring of the crosses between the most resistant and the two most susceptible. The three crosses were used to develop some molecular genetic markers associated with stem rust resistance using SSR and STS protocols. The results indicated that the presence of two positive markers out of the three SSR and three STS primers which used in this study. Sr2 (SSR) and Sr25 (STS) primers produced positive markers at fragment sizes of 120 and 130 bp, respectively, for stem rust resistance that could be considered as reliable markers for stem rust resistance in bread wheat *(Ttriticum aestivum)*.

Key words: Wheat, Stem rust, Stem rust and yield related traits, Molecular markers for stem rust, SSR & STS markers.

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LIST OF ABBREVIATION

AFLP	Amplified Fragment Length Polymorphism
ARC	Agricultural Research Center
CIMMYT	International Center for Maize and Wheat Improvement
DGGE	Denaturing Gradient Gel Electrophoresis
ICARDA	International Center for Agricultural Research in the Dry Areas
MAS	Marker Assisted Selection
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Loci
RAPD	Randomly Amplified Polymorphism DNA
RFLP	Restriction Fragment Length Polymorphism
STS	Sequence Tagged Sites
SSR	Simple Sequence Repeats

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important strategic cereal crop for the majority of the world populations. Wheat is one of the oldest and most important of the cereal crops in Egypt. The annual consumption of wheat grains in Egypt is about 12.4 million tons, while the annual local production is about 9.38 million tons/3.4 million faddan, while consumption is about 12.4 m.tons in 2014/2015 (gap: 3.02 m.tons). **(Agric. Economics and Statistics Department, Ministry of Agriculture, Egypt 2015).**

Therefore, the efforts of wheat breeders and geneticists must continue to increase the productivity of area unit to face the gap between production and consumption in Egypt, though grown under a wide range of climates and soils. The required yield increase may be achieved by developing high-yielding cultivars simultaneously, implementing improved cultural practices. The new improved cultivars must be resistant to serious diseases such as wheat rusts, tolerant to abiotic stresses namely; drought, salinity and heat, and should be stable in a broad spectrum of environments (having wide adoptability).

Stripe, leaf and stem rusts caused by *Puccinia striiformis, Puccinia triticinea* and *Puccinia graminis,* respectively are globally important wheat fungal diseases that cause significant grain yield losses. Stem rust can cause severe yield losses in susceptible cultivars of wheat in environments favorable for disease development (**Leonard and Szabo, 2005**). Stem rust resistance genes were successfully deployed in commercial cultivars worldwide from the middle 1950s, effectively controlling the disease. However, in 1999, a new race of stem rust, Ug99, also called TTKS, emerged in Uganda (**Pretoruis** *et al.*, **2000**). Later, it was also found in Kenya, Ethiopia and Yemen (**Singh** *et al.*, **2006**). More recently, Ug99 has spread to major wheat production areas of the Middle East, such as Iran, Afghanistan, India, Pakistan, Turkmenistan, Uzbekistan and Kazakhstan. The possible route of stem rust spread is proposed as East Africa – Middle East – West Asia – South Asia (**Singh** *et al.,* **2006**). Use of resistance wheat cultivars is the most economic and environmentally safe way to reduce crop losses from rust diseases. However, understanding the genetic behavior of wheat resistance to these diseases is essential for deciding the breeding method that maximizes the genetic improvement of these characters **(Shehab El-Din** *et al.,* **1991)**.

Wheat resistance to rusts has been documented to be a simple inherited character governed by one, two or a few number of major gene pairs **(Dyck, 1991)** and **(Bai** *et al.,* **1997)**. Meanwhile, several investigators indicated that resistance is a quantitative character controlled by many genes as well as the prevailing environmental conditions, **(Shehab El-Din** *et al.,* **1991), (Yadav** *et al.,* **1998) and (Nawar** *et al.,* **2010)**.

Molecular markers are useful tools to study the genetic variations **(Röder** *et al.,* **2002)**. It offers the simplest and fastest method for detecting a great number of genomic markers in less period of time **(Edwards** *et al.,* **1992)**. **Michelmore** *et al.* **(1991)** developed the F_2 plants population to the highest and the lowest extremes for the development of markers needed for marker-assisted selection. Marker-assisted selection program was progressed markers in several crop plants such as wheat **(Penner** *et al.,* **1996)**, durum wheat **(Wang** *et al.,* **1995)**.

Several of the Sr genes derived from wild relatives present on Robertsonian translocations or small chromosomal introgression segments have been relied upon in for breeding programs and have been deployed commercially including *Sr*24, 25, 31, 36 ,38, 5 and Sr1RAmigo **(Singh, 2008)**. Sr2 has provided durable resistance since its introduction to hexaploid wheat in the 1920s (**McFadden, 1930**).

The objectives of this study are to screen the response of three contrasting parents and their F1 and F2 plants to stem rust by recording the rust reaction and some related traits to stem rust. Detect some molecular genetic markers associated with stem rust using SSR & STS markers.

REVIEW OF LITERATURE

1. Stem rust pathogen and disease cycle.

On barberry, *P. graminis* starts its life cycle by producing black thick walled, diploid teliospores that keep *P.graminis* dormant overwinter **(Roelfs, 1985)**. After karyogamy, which forms a diploid nucleus from the fusion of two haploid nuclei, meiosis begins and results in four haploid basidiospores. In spring, each teliospore germinates to produce two identical thin walled haploid basidiospores **(Roelfs, 1985; Leonard and Szabo, 2005).** Mature basidiospores are carried by air currents to reinfect barberry. Basidiospores germinate and form a haploid mycelium growing on the leaf surface. From the mycelium, pycnia are produced on the upper leaf surface of barberry. Pycnia produce receptive hyphae that serve as female gametes and pycniospores served as male gametes **(Roelfs, 1988)**. Pycniospores are produced in honeydew that is attractive to insects, and rain splashing helps disperse pycniospores. When pycniospores are paired with receptive hyphae, cross-fertilization occurs successfully and dikaryotic mycelium forms. Then a cup-shaped dikaryotic aecium forms to release aeciospores. Normally aeciospores stalked in chains produced on barberry are transported by wind to start a new disease cycle in susceptible wheat cultivars **(Roelfs, 1985; Leonard and Szabo, 2005).**

In wheat, rust infection mainly occurs on stems and leaf sheaths. Within two weeks after inoculation, a brick-red structure, called a rust pustule also known as a uredium containing urediospores, appears at the point of inoculation. In heteroecious rusts, urediospores can reproduce themselves and re-infect wheat multiple times **(Leonard and Szabo, 2005)**, which can lead to severe damage on wheat production. In a later developmental stage, another type of spore called teliospore, which is a black overwintering spore, is produced in telia **(Cummins and Hiratsuka, 2003)** to conclude the disease cycle of stem rust in wheat and to start a new life cycle in barberry **(Figure 1)**.