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## Research Article

# Common Ancestry of Egyptian *Puccinia striiformis* Population Along with Effective and Ineffective Resistance Genes

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### Abstract

**Background and Objective:** Stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) has been constantly reported as an increasing problem with repeated cases of worldwide invasions, causing great losses of wheat production. This study aimed to determine the common ancestry of the Egyptian population of *Pst* races and effective/ineffective resistance genes. **Materials and Methods:** Rusted wheat samples collected across Egyptian provinces were processed for race analysis. Single uredinial isolates of *Pst* were inoculated to 8 day-old seedlings of a core set of the World/European differential hosts and Avocet near-isogenic lines corresponding to 14 stripe rust resistance genes (*Yr*), i.e., *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr17*, *Yr25*, *Yr27*, *Yr32* and *Yr Sp*. **Results:** Virulence phenotyping revealed the common ancestry of the current population of *Pst* belonging to three races i.e., Triticale aggressive (virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr10*), *Pst53* (virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr25*) and *Pst52* (virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*). The effective genes for Triticale aggressive race were *Yr1*, *Yr3*, *Yr4*, *Yr9*, *Yr17*, *Yr25*, *Yr27*, *Yr32*, *YrSp*, while effective genes for *Pst52* race were *Yr1*, *Yr3*, *Yr4*, *Yr10*, *Yr17*, *Yr32*, *YrSp* and for the race *Pst53* were *Yr1*, *Yr3*, *Yr4*, *Yr9*, *Yr10*, *Yr17*, *Yr27*, *Yr32*, *Yr Sp*. **Conclusion:** The Triticale aggressive race was first detected in Egypt, while the Warrior race was not found. Resistant genes for the three detected races should be considered in breeding programmes.

**Key words:** Wheat, *Puccinia striiformis*, virulence phenotype, exotic incursions, resistance genes

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**Competing Interest:** The author has declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is a widespread disease across major wheat growing regions with diverse cropping systems, growing seasons and germplasm characteristics<sup>1-3</sup>. Resulting losses have been estimated to be at least 5.5 million t per year at worldwide level<sup>4</sup>. Over the last decade, a series of regional outbreaks of stripe rust epidemics have been reported worldwide, including Central and West Asia and East and North Africa (www.globalrust.org). High disease pressure was observed in 2009 and onward in North Africa, particularly in Morocco<sup>5</sup>. Since 2010, stripe rust was widely spread in East Africa causing economic losses in low-input farming system<sup>6</sup>. Widespread epidemics were observed in Tajikistan in 2010 and later on in Uzbekistan and other countries of Central Asia<sup>7</sup>. In 2010, high disease prevalence was observed in 2010 in Syria and Lebanon imparting economic losses<sup>8</sup>. In Europe, the established *P. striiformis* population has largely been replaced since 2011 by distinct new lineages, generally known as Warrior, Kranich<sup>9</sup> and another lineage associated with epidemics on triticale, particularly in Scandinavia<sup>10</sup>. In Egypt, evidence of increased aggressiveness of the disease has been reported<sup>11,12</sup>; this may be due to the pathotypic evolution of the races that are able to overcome the resistance sources of wheat<sup>12,13</sup>, where new races have recently emerged, causing increased epidemics on multiple wheat varieties<sup>12</sup>.

The evolution of virulence to different host resistance genes has been recently documented through the comprehensive monitoring of *P. striiformis* virulence phenotypes<sup>12,14-16</sup>. In Egypt, most of the predominant races up to 2011 were typical of the NW-European genetic group although races of atypical virulence phenotypes were also observed in several countries and years<sup>11,12,14,17</sup>. The latter often had distinctly different molecular characteristics and were interpreted as exotic to the Egyptian population<sup>12</sup>. Most exotic races up to 2011 only had a minor impact on wheat in Egypt due to the presence of resistance preventing their establishment on most of the deployed host varieties<sup>11,12</sup>.

Wheat stripe rust has been controlled to a large extent via ongoing and large-scale breeding efforts to improve disease resistance, which is the most economical means and sometimes the only available option<sup>6</sup>. Successful deployment of resistant crop varieties at larger scales and in different regions would, however, require a better understanding of pathogen diversity for virulence across regions<sup>18</sup>. The virulence structure of the pathogen has often been described at the country or regional scales<sup>2,12,16,19,20</sup>. In Egypt, attempts to link virulence and race structure with the recent regional stripe

rust epidemic outbreaks in different parts of the world are missing. In summary, understanding of the population structure of *P. striiformis* in Egypt is fragmented and additional analysis and documentation are needed. The main objective of the study was to determine the common ancestry of the current Egyptian population of *P. striiformis*, in beside the effective and ineffective resistance genes.

## MATERIALS AND METHODS

**Sample collection:** Stripe rust-infected leaves were collected from wheat rust trap nurseries, breeding trials and commercial wheat fields across Egyptian provinces during 2016-2018. The number of samples varied across years and provinces, partly associated with the infection situation and sampling effort in a particular region, which reflected the national resources allocated for the survey or the significance of yellow rust disease in different years.

**Race multiplication:** Single pustules were separately inoculated on seedlings (8 day old) of the highly susceptible wheat cultivar Morocco. The inoculated seedlings were misted with water and incubated in a dark dew chamber at 10°C for 24 h under high relative humidity. After incubation, the inoculated plants were transferred to a growth greenhouse conditioned at a temperature of 13±2°C, relative humidity up to 100% under a light intensity of 100 µmol m<sup>-2</sup> sec<sup>-1</sup> in a photoperiod of 16 h light and 8 h dark<sup>21</sup>. Fresh spores were collected 15-20 days after inoculation of each single uredinial isolate to be subjected for virulence phenotyping.

**Virulence phenotyping:** Virulence phenotyping was made on a set of 30 wheat differential lines, which for the most common races of virulence (*v*) corresponding to 14 stripe rust resistance genes (*Yr*), i.e., *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr17*, *Yr25*, *Yr27*, *Yr32* and *YrSp*. Avocet S (AvS) served as a susceptible control. The differential lines consisted of a core set of the World/European differential sets (W/E)<sup>22,23</sup> and Avocet near-isogenic lines (NILs)<sup>15,16,24</sup>. Seedlings of differential lines (8 day-old) were inoculated with single-uredinial isolates of *Pst* and kept in a growth greenhouse conditioned following previously method of Stubbs<sup>21</sup>. First seedling leaf was considered for virulence phenotyping on a 0-9 scale<sup>25</sup>, where infection type 7-9 were generally considered to reflect compatibility (virulence) and 0-6 incompatibility (avirulence). The phenotype for virulence (*v*) and avirulence (*Av*), corresponding to the individual resistance genes were in most cases deduced from the infection types on two to three differential lines carrying the considered *Yr* gene.

**Naming system for *Pst* races:** The common ancestry profile/phenotype was inferred based on the overall virulence combination and each distinct virulence phenotype was considered a distinct race. Common names of *Pst* races have been assigned according to their genetic lineage identified through their molecular genotyping in comparison with the worldwide defined genetic groups<sup>16,20</sup>.

## RESULTS

Single-uredinial isolates of *Pst* derived from the stripe rust samples were virulence phenotyped on differential lines with 14 resistance genes *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr17*, *Yr25*, *Yr27*, *Yr32* and *YrSp*. Virulence phenotyping (Table 1) revealed the common ancestry of the current Egyptian population of *Pst* races belonging to three common races i.e., Triticale aggressive (virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr10*), *PstS3* (virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr25*) and *PstS2* (virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*). No collections were found with the Warrior race, that has virulence to *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr9*, *Yr17*, *Yr25*, *Yr32*, *YrSp*.

**Effective and ineffective resistance genes:** The genes *Yr1*, *Yr3*, *Yr4*, *Yr9*, *Yr17*, *Yr25*, *Yr27*, *Yr32*, *YrSp* were effective for the race Triticale aggressive, while *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr10* were ineffective ones. The effective genes for *PstS2* race were *Yr1*, *Yr3*, *Yr4*, *Yr10*, *Yr17*, *Yr32*, *YrSp*, while ineffective genes consisted of *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*. The genes *Yr1*, *Yr3*, *Yr4*, *Yr9*, *Yr10*, *Yr17*, *Yr27*, *Yr32*, *YrSp* represented the effective genes for the race *PstS3*, while *Yr2*, *Yr6*, *Yr7*, *Yr8* and *Yr25* were ineffective ones. The effective resistance genes for the three *Pst* races were *Yr1*, *Yr3*, *Yr4*, *Yr17*, *Yr32* and *YrSp*, while *Yr2*, *Yr6*, *Yr7* and *Yr8* occupied ineffective resistance (Table 2).

## DISCUSSION

Wheat stripe rust pathogen *Puccinia striiformis* is a biotrophic basidiomycete that has a capacity for long-distance migration by airborne spores<sup>16,26,27</sup>. A number of invasions originating from distant geographical areas have been

reported, either as an incursion to a region where it was previously absent (e.g., North and South America, Australia and South Africa) or as re-emergence of new virulent races with increased aggressiveness and tolerance to high temperatures in North America, Australia and North Africa<sup>1,20,28</sup>. The pathogen has a recombinant population structure and a high diversity in the Centre of diversity in the Himalayan and near-himalayan region but a clonal population structure in Europe, America and Australia<sup>10,20</sup>. This study revealed the common ancestry of the current Egyptian population of *P. striiformis*, that provides evidence of a gradual replacement of the pre-existing clonal population of the stripe rust isolates.

In the present study, the current Egyptian population of *Pst* races showed the common ancestry belonging to three races i.e. Triticale aggressive (virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr10*); *PstS3* (virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr25*); and *PstS2* (virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*). The Triticale aggressive race of non-Egyptian origin, first detected in Egypt in this study was most similar to populations in the Middle East/Central Asia<sup>3</sup>. No collections were found with the Warrior race, that has virulence to *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr9*, *Yr17*, *Yr25*, *Yr32*, *YrSp*. Virulence relatedness revealed some evolutionary patterns of *Pst* races. However, molecular patterns indicated their common ancestry since the new races

Table 1: Common ancestry of Egyptian *Pst* races identified in this study based on virulence phenotyping on W/E differential and Avocet NILs corresponding to 14 *Yr* genes

<i>Yr</i> gene	<i>Pst</i> races/Virulence phenotype		
	Triticale aggressive	<i>PstS2</i>	<i>PstS3</i>
* <i>Yr1</i>	Av	Av	Av
* <i>Yr2</i>	v	v	v
* <i>Yr3</i>	Av	Av	Av
* <i>Yr4</i>	Av	Av	Av
* <i>Yr6</i>	v	v	v
* <i>Yr7</i>	v	v	v
* <i>Yr8</i>	v	v	v
* <i>Yr9</i>	Av	v	Av
* <i>Yr10</i>	v	Av	Av
* <i>Yr17</i>	Av	Av	Av
* <i>Yr25</i>	Av	v	v
* <i>Yr27</i>	Av	v	Av
* <i>Yr32</i>	Av	Av	Av
* <i>YrSp</i>	Av	Av	Av

\*New record on Avocet lines carrying single *Yr* gene overplus W/E differential set, v: Virulence, Av: Avirulence

Table 2: Effective and ineffective *Yr* genes for *Pst* races identified in this study

<i>Pst</i> race	Effective <i>Yr</i> genes	Ineffective <i>Yr</i> genes	Effective genes for three races	Ineffective genes for three races
Triticale aggressive	<i>Yr1</i> , <i>Yr3</i> , <i>Yr4</i> , <i>Yr9</i> , <i>Yr17</i> , <i>Yr25</i> , <i>Yr27</i> , <i>Yr32</i> , <i>YrSp</i>	<i>Yr2</i> , <i>Yr6</i> , <i>Yr7</i> , <i>Yr8</i> , <i>Yr10</i>	<i>Yr1</i> , <i>Yr3</i> , <i>Yr4</i> , <i>Yr17</i> , <i>Yr32</i> , <i>YrSp</i>	<i>Yr2</i> , <i>Yr6</i> , <i>Yr7</i> , <i>Yr8</i>
<i>PstS2</i>	<i>Yr1</i> , <i>Yr3</i> , <i>Yr4</i> , <i>Yr10</i> , <i>Yr17</i> , <i>Yr32</i> , <i>YrSp</i>	<i>Yr2</i> , <i>Yr6</i> , <i>Yr7</i> , <i>Yr8</i> , <i>Yr9</i> , <i>Yr25</i> , <i>Yr27</i>		
<i>PstS3</i>	<i>Yr1</i> , <i>Yr3</i> , <i>Yr4</i> , <i>Yr9</i> , <i>Yr10</i> , <i>Yr17</i> , <i>Yr27</i> , <i>Yr32</i> , <i>YrSp</i>	<i>Yr2</i> , <i>Yr6</i> , <i>Yr7</i> , <i>Yr8</i> , <i>Yr25</i>		

appear to be genetically distinct and may represent an exotic introduction rather than a mutation in isolates of the old races<sup>12</sup>. These results emphasized the fact that, despite the continuous development and deployment of host genotypes with resistance to the prevalent pathogen population, invasions may initiate new disease epidemics<sup>18</sup>.

In this study, only one exotic race of *P. striiformis* was detected, termed "Triticale aggressive". The Triticale aggressive race first detected in Europe in 2006 on the island Bornholm in the Baltic Sea, became prevalent over large areas within one or a very few years<sup>10</sup>. The Triticale aggressive race was expected by initiating susceptible reactions on Egyptian wheat varieties previously known as resistant, probably due to the absence of resistance genes in these varieties such as *Yr1*, *Yr3*, *Yr4*, *Yr9*, *Yr17*, *Yr25*, *Yr27*, *Yr32*, *YrSp* that exhibited resistance for this race in this study. Triticale aggressive race had virulence phenotype that differed only by *Yr10* virulence from pre-2011 Egyptian races. The aggressive, high temperature-adapted strain *PstS2*, identified in this study, was detected between 2000 and 2004 in several European countries but often at low frequencies<sup>15,28</sup>. Another high temperature-adapted strain, *PstS3* (also often referred to as 6E16), detected in this study, carrying a low number of virulences was mainly present in southern Europe and only occasionally in the North<sup>15,17</sup>. The Triticale aggressive race may have established in Egypt after the 2011/12 wheat cropping season, where stripe rust epidemics on wheat were present in many growing areas. The races *PstS2* and *PstS3* may have remained in Egypt due to the absence of resistance genes such as *Yr1*, *Yr3*, *Yr4*, *Yr17*, *Yr32*, *YrSp* in Egyptian varieties that provided effective control of these races<sup>15,28,29</sup>. This provided an open niche for invasions from Europe as it is well documented that *P. striiformis* may spread long distances through wind dispersal of spores<sup>18,26</sup>. Natural recombination would require asynchrony in the prevalence, susceptibility and phenology of alternate host (*Berberis* spp.), as well as the primary cereal host. This was not the case in the US Pacific Northwest<sup>30</sup>, likewise in Europe<sup>16</sup> and it is most probably not the case in Egypt, where *Berberis* spp. has so far not been reported in Egypt. Concordance of virulence structure and establishment of certain races with regionally deployed host resistance emphasized the role of host selection on pathogen virulence structure and emphasized the need for greater regional and local diversification of host resistance.

### CONCLUSION

The present study is the first demonstration of the common ancestry of *Pst* population in Egypt based on the

overall virulence combination to stripe rust resistance genes. Virulence phenotyping data of *Pst* isolates based on the W/E differential and Avocet NILs, corresponding to 14 *Yr* genes (*Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr17*, *Yr25*, *Yr27*, *Yr32*, *YrSp*), revealed the common ancestry of the current Egyptian population of *Pst* belonging to three races i.e., Triticale aggressive, *PstS3* and *PstS2*. The Triticale aggressive race of non-Egyptian origin was first detected in Egypt, while no collections were found with the Warrior race. The effective resistance genes for the three races *Yr1*, *Yr3*, *Yr4*, *Yr17*, *Yr32* and *YrSp*, could be incorporated in breeding programmes of resistant wheat varieties.

### SIGNIFICANCE STATEMENT

This study discovered the common ancestry of the current Egyptian population of stripe rust pathogen that can be beneficial for flexible access to a complete race phenotype data for virulence structure of *P. striiformis*. This study will help the researchers to uncover the critical areas of incomplete race phenotype data that many researchers were not able to explore. Thus, a new theory on the effectiveness of stripe rust resistance genes may be arrived at.

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