Virulence and Diversity of Wheat Stripe Rust Pathogen in Egypt

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Abstract: Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* is a major disease on wheat in Egypt and worldwide. Virulence of *Puccinia striiformis* f. sp. *tritici* isolates, collected, were identified on seedlings of the wheat differential genotypes in the greenhouse condition. Thirteen and seven physiological races were identified in 2012/2013 and 2013/14 respectively. The Path types were identified during two successive seasons. In 2012/13 they were *i.e.* 0E0, 6E0, 2E0, 2E16, 4E0, 4E4, 6E5, 6E20, 18E16, 34E16, 34E20, 38E20, 70E4 and in 2013/2014 *i.e* 0E0, 2E0, 2E8, 4E0, 6E116, 70E20 and 128E28 were identified during growing seasons. Race 0E0 was the most frequent one followed by 2E0, 4E0 and 6E4. The obtained results showed that *Yr's*: 1, *5,* and *SP* were the most effective during growing seasons since no virulence were recorded on either one. On the other hand, *Yr's 7, 6* and *6* were attacked by a high number of races. Regarding evaluation of certain stripe rust wheat monogenic lines and Egyptian wheat varieties under the stress of both greenhouse and field conditions, the obtained results indicated that *Yr1, 5,10,15, 17, 32* and *SP* were resistant at seedling and adult stages. Genes such as *YrA* and *Yr18* were resistant only at adult plant resistance (APR), were testing the released wheat cultivars under natural **conditions**, Sakha 93 and Sids 12 were found infected. These results would serve as a fruitful tool in the wheat breeding program directed for disease resistance.

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Keyword: Wheat, yellow rust, physiological races, and virulence/a virulence formula.

1. Introduction

Wheat (*Triticum aestivum* L.) stripe rust incited by *Puccinia striiformis* f. sp. *tritici* is an important disease worldwide. Low temperate and high relative humidity are the suitable factors to the wide distribution of the disease (**Stubbs. 1988; Johnson, 1988 and Denial, 1994**).

Screening of varieties against stripe rust is a regular activity due to the dynamic evolutionary nature of the pathogen. The fungal pathogen evolves into new races quickly through mutation and somatic hybridization (**Stubbs, 1985**).

In Egypt it is a sporadic disease because it appears in same years in Near and Middle East areas. But starting from 1990's it became familiar due to it's continuous appearance. (**Abu El-Naga, 2001**). Screening of varieties against stripe rust is a regular activity due to the dynamic evolutionary nature of the pathogen. The fungal pathogen evolves into new races quickly through mutation and somatic hybridization **(Stubbs, 1985).** Being airborne, local races can migrate to other areas and quickly become regionally and often globally predominant. Thus, virulence has been reported for many Yr genes worldwide. However, virulence for certain genes or gene combinations may still be absent regionally **(Singh *et al.,* 2002).**

The disease was recognized as epidemics i.e. 1967/68 on wheat varieties Giza 144 at Manzala district, 1995/96, 1997/98 on Sakha 69, Giza 163, Gemmeiza 1 and most of the commercial varieties especially the long spiked ones, at the Northern governorates in particular a little bet cases of epiphytotics were recorded during 1999/2000 (**Abdel-Hak, *et al*., 1972; El-Daoudi, *et al*., 1996 and Abu El-Naga, *et al*., 1997, 1999 and 2001).**

Seedling resistance is usually race specific and can be recognized by its characteristic resistance type at all plant stage **(Hong and Singh 1996).** Adult resistance can be either race specific or race non-specific and it usually better recognized after the seedling stage **(Jonhson, 1988). Singh (1992)** reported that adult plant resistance gene *Yr18* is involved in durable resistance of several bread wheat cultivars including the North American cultivar Anza. Genes conferring adult plant resistance are known to occur in wheat.

The objective of this study was to identify the virulence analysis and race diversity of the stripe rust pathogen in Egypt during the period from 2011 to 2014 and to determine the effectiveness of the resistance genes against the rust population. Evaluated monogenic lines and some Egyptian varieties against stripe rust disease on wheat at both seedling and adult stages.

2. Material and Methods

In this experiment stripe rust population were clarified for their virulence pathotypes and pathogenic variations using at seedling stage the method was the identification of stripe rust physiologic races were using the World and European group of wheat differential varieties as proposed by **Johnson *et al.* (1972)** in Table (1).

Isolate collected of 132 samples (leaves and spikes) were obtained at heading stage from differential cultivars and different location *i.e.* (Kafrelsheikh, Dakahliya, Gharbiya and Damietta) the collected samples were purified and multiplied on susceptible i.e. Morocoo the methods adopted by **Stubbes (1988)** urediniospores were used to inoculate in 7-10 days-old seedling of the yellow rust differential genotypes and commercial cvs. Inoculated plants were placed for 24 h in a dew chamber at 10 °C then transferred to the permanent cabinets at day/night rhythm was 8/16h temperature was adjusted at 15°C light intensity 7500 lux.

Infection types (IT) data of the plant-pathogen interaction were recorded 15-20 days after inoculation using the infection type equal to or higher than 7 were considered susceptible and those less than 7 were considered resistant in turn the aggressive races were considered virulent/a virulent. Race identification and virulence formula were recorded 15-20 days after developed races of *Puccinia striiformis* f. sp. *tritici* were determined by IT based on the 0 to 9 scale adopted by **Mc Neal *et al.* (1971)**, virulence and gene efficacy were applied according to the method adopted by **Green (1965).**

Regarding evaluation of isogenic lines and teen bread wheat cultivars against Pst infection under field condition, one row for each of the isogenic lines and cultivars were sown in 1 m long and 30 cm apart highly susceptible cultivars were sown around the experiment i.e. Morocco. The disease severity of infection 0 to 100% and infection type (R and S) were recorded at heading stage using modified cob scale **(Peterson, 1948).**

Table (1).*Differential wheat genotypes used to identify patho types of stripe rust incited by *Puccinia striiformis* f. sp. *tritici* in Egypt.

Differential cultivars	Abbreviation	Decanery value	Resistance gene	Type
GI. World differential set[1]				
Chinese 166	Ch	(2^0) =1	*Yr1*	winter
Lee	Lee	(2^1) = 2	*Yr7*	spring
Heines Kolben	HK	(2^2) = 4	*Yr2 Yr6*	spring
Vilmorin 23	V23	(2^3) = 8	*Yr3*	winter
Moro	Mo	(2^4) = 16	*Yr10*	winter
Strubes Dickkopf	Std	(2^5) = 32	*SD*	winter
Suwon 92 × Omar	Su	(2^6) = 64	*SU*	winter
Clement	Cl	(2^7) = 128	*Yr2 Yr9*	winter
Tirticum spelta Album	Sp	(2^8) = 256	*Yr5*	spring
GII. European Differential set[1]				
Hybrid 46	H46	(2^0) =1	*Yr 4*	winter
Reichersberg 42	R42	(2^1) = 2	*Yr(7)*	winter
Heines Peko	Pe	(2^2) = 4	*Yr2 Yr (6)*	spring
Nord Desprez	No	(2^3) = 8	*Yr(3)*	winter
Compair	Com	(2^4) = 16	*Yr8*	spring
Carstens V	CV	(2^5) = 32	*YrCV*	winter
Spaldings Prolific	Spa	(2^6) = 64	*YrSP*	winter
Heines VII	HVII	(2^7) = 128	*Yr2*	winter

*** Johnson *et al.* (1972**).

3. Results

Identification of physiologic race:

For a period of two consecutive years, leaf samples were collected from bread wheat cultivars, as well as from differential cultivars grown under field conditions in four location *i.e.* (Kafrelsheikh, Dakahliya, Gharbia and Damietta) province of Egypt Single-uredinial isolates of P. *striiformis* were characterized for physiologic races. The results obtained from this study showed that there were identified 20 physiologic races during the course of this study.

In the first season (2012/13), Data in Table (2) revealed the occurrence of 13 races of stripe rust (0E0, 6E0, 2E0, 2E16, 4E0, 4E4, 6E4, 6E5, 6E20, 18E16, 34E20, 38E20 and 70E4). The race 6E4 was the most frequent one (16.66%) followed by race 70E40 (13.33 %), race 38E20 (11.66%), race 4E4 (10.00 %), race

0E0 and 6E5 (8.33%). The least ones in this regard were races 18E16 (3.33%) and race 34E20 (1.66%).

In the second season (2013/14**),** Data in Table (3) revealed the occurrence of 7 races of stripe rust *i.e.* 0E0, 2E0, 2E8, 4E0, 6E16, 70E20 and 128E28. The most frequent race was 2E0 (37.5%) followed by race 2E8 (11.66%), race 0E0 and 4E0 (12.5 % each), race 6E16 (10.00 %), race 70E20 (7.5%). The race 128E28 was the least one in this regard by 5%.

Table. (2) Wheat yellow rust patho type identify their frequency and virulence in Egypt during 2012/2013.

No.	Races	No. of isolates	Frequency (%)	Virulence/avirulence
1	0E0	5	8.33	/1,7,6,3,10,SD,SU,9,5,4,(7),(6),(3),8,CV,SP,2
2	6E0	3	5.00	7,6/1,3,10,SD,SU,9,5,4,(7),(6),(3),8,CV,SP,2
3	2E0	3	5.00	7/1, 6,3,10,SD,SU,9,5,4,(7),(6),(3),8,CV,SP,2
4	2E16	4	6.66	7,8/1,6,3,10,SD,SU,9,5,4,(7),(6),(3),CV,SP,2
5	4E0	4	6.66	6/1,7,3,10,SD,SU,9,5,4,(7),(6),(3),8,CV,SP,2
6	4E4	6	10.00	6,(6)/1,7,3,10,SD,SU,9,5,4,(7),(3),8,CV,SP,2
7	6E4	10	16.66	7,6,(6)/1,3,10,SD,SU,9,5,4,(7),(3),8,CV,SP,2
8	6E5	5	8.33	7,6,4,(6)/1,3,10,SD,SU,9,5, (7),(3),8,CV,SP,2
9	6E20	3	5.00	7,6,(6),8/1,3,10,SD,SU,9,5,4,(7),(3),CV,SP,2
10	18E16	2	3.33	7,10,8/1,6,3,SD,SU,9,5,4,(7),(6),(3),CV,SP,2
11	34E20	1	1.66	7,SU,(6),8/1,6,3,10,SD,9,5,4,(7),(3),CV,SP,2
12	38E20	7	11.66	7,6,SD,(6),8/1,3,10,SU,9,5,4,(7),(3),CV,SP,2
13	70E4	8	13.33	7,6,SU,(6),4 /1,3,10,SD,9,5,4,(7),(3),8,CV,SP,2

Table (3). Wheat yellow rust pathotype identify their frequency and virulence in Egypt during 2013/2014.

No.	Races	No. of isolates	Frequency (%)	Virulence/avirulence
1	0E0	5	12.5	/1,7,6,3,10,SD,SU,9,5,4,(7),(6),(3),8,CV,SP,2
2	2E0	16	37.5	7/1,6,3,10,SD,SU,9,5,4,(7),(6),(3),8,CV,SP,2
3	2E8	6	15	7,(3)/1,6,3,10,SD,SU,9,5,4,(6),8,CV,SP,2
4	4E0	5	12.5	6/1,10,7,6,3, SD,SU,9,5,4, (6),(3),8,CV,SP,2
5	6E16	4	10	6,(7),8 /1,2,7,6,3, SD,SU,9,5,4, (6),(3),8,CV,SP,2
6	70E20	3	7.5	7,6,SU,(6),8 /1,3,10,SD,9,5,4,(7),(3),CV,SP,2
7	128E28	2	5	9,(6),(3),8/1,3,10,SD,4,CV,2,6,7,SU, 5,(7),SP,2

Frequency of Virulence:

Virulence Frequency of the causal organism (*Puccinia striiformis* f. sp. *tritici*) of wheat stripe rust was studied using rust samples collected. Virulence was tested against 17 differential hosts for stripe rust resistance.

The results presented in Table (4), the obtained results revealed that the lowest frequencies of virulence were recorded with *Yrs* i.e. *Yr1, Yr3,, Yr9, Yr5, Yr(3), Yr CV, Yr SP* and *Yr2* (0 %) in an ascending order. On the other hand *Yr6, Yr7, Yr(6)* and *Yr8* arranged according to their virulence frequency, 76.66, 71.66, 66.66 and 28.33, respectively during 2012/13.

The obtained results revealed that the lowest frequencies of virulence were recorded with *Yrs* i.e. *Yr1, Yr 9, Yr10, Yr5, Yr 4, Yr(7), YrCV, Yr SP* and *Yr2*(0 %) in an ascending order. On the other hand *Yr7, YrSU, Yr6, Yr8* and *Yr(6)* arranged according to their virulence frequency, 71.00, 58.00, 30.00, 22.00 and 20.00, respectively during 2013/14.

It was observed that Yr1, Yr5 and Yr SP conferred the highest efficacy for resistance against all isolates at the three seasons exhibiting 100% gene efficacy.

Response of isogenic lines and some Egyptian wheat cultivars against stripe rust at both seedling and adult stages in three locations:

Data presented in table (5) showed that resistance in both seedling and adult stages *i.e. Yr 1, Yr 5, Yr 10, Yr 15, Yr 32* and *Yr SP* during the two seasons. While *Yr A, YrSK* and $Yr18^{+}$ were susceptible in seedling stage while, were resistant at adult plant resistance (APR). On the other hand *Yr6, Yr (6), Yr7, Yr8* and *Yr9* were susceptible in both seedling and adult stages. The presented data also indicated that, the tested ten Egyptian wheat cultivars were susceptible at seedling stage while, the tested wheat cultivars i.e. Misr- 1, Misr-2, Gemmeiza-10 and Giza -168 showed resistance at adult stage during the two seasons against the used field cultures. On the other hand Gemmeiza 11, Sids 12 and Sakha 93 were susceptible in both seedling and adult stages.

Table (4). Stripe rust effective genes and efficacy action within physiologic races in 2012/13 and 2013/14 growing seasons

Yr's	2012/2013 growing seasons			2013/14 growing seasons		
	No. of virulent isolates	Virulent Frequency %	Gene efficacy %	No. of virulent isolates	Virulent frequency %	Gene efficacy %
1	0	0.00	100.00	0	0.00	100.00
7	46	75.40	24.59	25	60.97	39.02
6	39	63.93	36.06	12	29.26	70.73
3	0	0.00	100.00	0	18.00	82.00
10	2	3.27	96.72	0	0.00	100.00
SD	7	11.47	88.52	0	0.00	100.00
SU	9	14.75	85.24	3	7.31	92.68
9	0	0.00	100.00	2	4.87	95.12
5	0	0.00	100.00	0	0.00	100.00
4	5	8.19	91.80	0	0.00	100.00
(7)	0	0.00	100.00	4	9.75	90.24
(6)	41	67.21	32.78	5	12.19	87.80
(3)	0	0.00	100.00	9	21.95	78.04
8	17	27.86	72.13	9	21.95	78.04
CV	0	0.00	100.00	0	0.00	100.00
SP	0	0.00	100.00	0	0.00	100.00
2	0	0.00	100.00	0	0.00	100.00

*SD = Strubs Dickhof, SU = Suan × Omar, CV= Carsten V, SP = Splding prolific.

Table (5). Reaction of differential genotypes to yellow rust in Northern Delta in Egypt over two growing seasons (2012-2014)

Genotypes	Yr gene	Season / reaction							
		2012/2013				2013/2014			
		Seedling stage	Adult stage			Seedling stage	adult stage		
			Kafrelsheikh	Dakahliya	Gharbia		Kafrelsheik	Dakahliya	Gharbia
Yr1/6*Avocet S	*1*	0;	0	TrR	0	0;	0	0	0
Yr5/6*Avocet S	*5*	0;	0	0	0	0;	0	0	0
Yr6/6*Avocet S	*6*	9	40S	50S	50S	9	50S	50S	50S
Yr7/6*Avocet S	*7*	9	50S	70S	70S	9	60S	70S	70S
Yr8/6*Avocet S	*8*	9	50S	80S	70S	9	50S	70S	80S
Yr9/6*Avocet S	*9*	8	20S	40S	30S	8	40S	30S	40S
Yr10/6*Avocet S	*10*	0;	TrR	5R	5R	0;	0	5R	5R
Yr15/6*Avocet S	*15*	0;	0	TrR	TrR	0;	0	TrR	TrR
Yr17/6*Avocet S	*17*	3	5MR	20MR	TrR	5	10MR	TrR	20MR
Yr18/6*Avocet S	*18*	9	20MS	10MS	10MS	9	10S	10MS	10MS
Yr27/6*Avocet S	*27*	6	5MS	TrMS	TrMS	7	20MS	TrMS	TrMS
Yr32/6*Avocet S	*32*	1	TrMR	0	0	2	0	0	0
YrSP/6*Avocet S	*SP*	0	0	0	0	1	0	0	0
Avocet-YrA	-	7	80s	60S	50S	7	70s	50S	60S
Avocet+YrA	*yrA*	6	70s	50S	50S	7	70s	50S	50S
YrSK/6*Avocet S	*SK*	9	0	0	0	9	0	0	0
Kalyansona	*2*	9	10MS	5MS	TrMS	9	20MS	TrMS	5MS
Anza	*A, 18*	7	TrMR	TrMR	TrR	7	10MR	TrR	TrMR
Jupateco 'R' (S)	*18+*	9	10MS	5MS	5MS	9	10MS	5MS	5MS
Jupateco (S)	-[c]	8	40MS	10MS	5MS	8	10S	5MS	10MS
Msir 1	-	6	0	0	0	7	5MR	0	0
Msir 2	-	6	TrR	0	0	7	10MR	0	0
Gemmeiza 10	-	7	0	0	0	7	10MR	0	0
Gemmeiza 11	-	7	0	0	0	7	10MS	0	0
Sids 12	-	6	20MS	10MS	10MS	7	40	10MS	10MS
Sids 13	-	7	TrMS	0	0	7	10MS	0	0
Sakha 93	-	8	30S	10S	10S	8	40S	10S	10S
Sakha94	-	8	5MR			8	10MR		
Giza168	-	7	5MR	0	0	8		0	0
Giza 171	-	7	5MS	0	0	8	5S	0	0

[a] seedling infection type follow a 0-9 scale as described in **Roelfs *et al.* (1992)**.,[b] The APR has two component, % rust severity based on the modified Cobb Scale (**Peterson *et al.*, 1948**) and response to infection as described by **Roelfs *et al.* (1992)**., [c] Unknown genes.

4. Discussion

The annual survey of wheat stripe rust conducted throughout three growing seasons of wheat crop in certain governorates of Egypt, gave evidence to the presence of wheat stripe rust and it's causal agent *Puccinia striiformis tritici* (syn. *Puccinia glumarum* Eriks. & Henn.). The annual survey for wheat diseases especially stripe rust is of a great importance because its ability to produce virulent physiologic races which threats the Egyptian wheat cultivars.

The obtained results gave evidence to the presence of 20 physiologic races of Pst during 2012to 2014 in two growing seasons. Similar results were recorded in Egypt by **Ashmawy 2005**, and **Youssef *et al*., 2003**, in other country, **Yahyaoui *et al*., 2002** identification 6E0 from sample collected from Syria 1999. Also this race was found in Egypt which indicated that the source of inoculasim maybe similar or the same.

Concerning the virulence of identified pathotypes and gene efficacy the obtained results indicated that non of the *Yr's5, 15* and SP could be attacked by the tested isolates. However *Yr's1,CV* proved to be effective genes from the perspective of their reaction on the differentials, the reverse was true with Yr SU quite different results was reported by Stubbs (1985) and **(Abu El-Naga *et al*., 1999 and shahin *et al.,* 2011)**.

Race 0E0 exhibited the highest frequency during the two seasons, race 0E0 and 6E4 exhibited the highest frequency during the two seasons, race 4E0 occurred throughout the two seasons. The rest of races appeared once a season each, with lower frequencies, similar results were reported by**, Youssef *et al.* (2003), Ashmawy (2005) and 2006 and 2007**, who confirmed the predominance of race 0E0, 4E0, 2E16 and 70E40, in Egypt. Similar results were reported in some countries other than Egypt *i.e.* the most predominant race was 0E0 it was recorded earlier at Saudi Arabia, Algeria and Morocco during 1990-1992. This was attributed to that the genes it attacks are not included in the differentials set, so, the differentials set must be supplemented with a complementary group of monogenic, Preferably a local Egyptian group. **Abu El-Naga, (2000) and Shahin (2008), Shahin, and Abu El-Naga, (2011).** Race 2E0 was recorded at IPO during 1980's as a dominant race within the Egyptian samples in addition to Turkey and Lebanon **(Stubbs, 1988).** It was virulent to lee (Yr7), similar results were reported in some courtiers other than Egypt because of the ability of rust urediospores to transport from one country to another and infect the susceptible cultivars. **(Yahyaoui *et al*., 2002, Hakim and Yahyaoui, 2003 and Wellings 2007).**

The obtained results indicated the good performance of Yr's 1, 9, and 5 and SP, since they couldn't be attacked all over the two seasons (100% efficacy) by either of the tested races. Likewise Yr's CV, (3), 4 and 10 occupied the second rank *i.e.* (>90%). *Yr's i.e. SD, SU*, and 8 came in the third rank (>80%). On the other hand, Yr' 6 (23.33), (7) (28.33), and (6) (33.33) were the least in this regard. These results were supported by those of **Abu El-Naga *et al.* (1999-2001), Youssef *et al*., (2003, 2006 and 2007). Singh (1992) and McIntosh (1992)** have indicated that the moderate level of durable adult plant resistance to stripe rust of the CIMMYT-derived US wheat cultivar Anza and winter wheat such as Bezostaja is controlled in part by the Yr18 gene. This gene is completely linked to the Lr34 gene. The level of resistance it confers is usually not adequate when present alone. However, combinations of Yr18 and 2-4 additional slow rusting genes results in adequate resistance levels in most environments (**Singh and Rajarm 1994**). The result showed that resistance in both seedling and adult stages *i.e. Yr 1, Yr 5, Yr 10, Yr 15, Yr 32* and *Yr SP* during the two seasons. While *Yr A, YrSK* and $Yr18^{+}$ were susceptible in seedling stage while, were resistant at adult plant resistance.

Genes such as Yr5 are previously known to show high level resistance to stripe rust in China, Iran, Turkey, North America and Africa (Macer, 1966; Wang et al., 1996; Zeybeck and Fahri, 2004; Chen 2005; Afshari, 2008). Furthermore, virulences to Yr5 and Yr15 genes rarely occur in wheat producing areas of the world (**Chen, 2005; Chunmei *et al*., 2008**).

Results obtained revealed that resistance in seedling Misr 1, Misr 2, Gemmeiza 10, Gemmeiza 11, and Sids 13 While susceptible in adult stage during the two seasons. These results were supported by those of **Abu El-Naga *et al*., (1999-2001), Youssef *et al*., (2003, 2006 and 2007), Ashmawy (2010).** Based upon, the obtained results could be useful for plant breeders if they incorporated such genes in a new genotype via hybridization made usually in breeding programs. They should put the pathogen genotype and environment in their considerations as two important variables in the pathogen: host: environment systems.

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