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IDENTIFICATION OF PHYSIOLOGICAL RACES AND VIRULENCE OF THE YELLOW RUST FUNGUS ON WHEAT IN EGYPT.

Shabana, Y. M.[*]; A. A. Shahin[**] and Hend A. Omar[**]

[*] **Plant Pathology Department, Faculty of Agriculture, Mansoura University, Egypt**

[**] **Plant Pathology Research Institute, Department of Wheat Diseases, ARC, Egypt**

ABSTRACT

Stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici* (Pst) is one of the most important wheat diseases in Egypt and worldwide. Isolates of (Pst) collected during 2008/2009 and 2009/2010 were identified in 2009/2010 and 2010/2011 growing seasons, respectively. Seven physiological races were identified in the first season i.e., 0E0, 4E0, 16E2, 16E128, 16E130, 60E153 and 60E177; while in the second season, 13 physiological races were identified i.e., 0E0, 6E0, 2E0, 2E16, 4E0, 4E4, 6E5, 6E20, 18E16, 34E16, 34E20, 38E20 and 70E4. Race 0E0 was the most frequent one followed by 4E0 and 6E4. Results obtained showed that *Yr*1*, Yr5, YrSU*, and *YrSP* were the most resistant genes against yellow rust in both growing seasons, while *Yr7*, *Yr6* and *Yr6*$^{+}$ were the most susceptible genes. These results are substantially important for wheat breeding programs for disease resistance.

Keyword: *Triticum aestivum*, stripe rust, physiological races, and virulence/avirulence formula.

INTRODUCTION

Wheat (*Triticum aestivum* L.) stripe rust incited by *Puccinia striiformis* f. sp. *tritici* (Pst) is an important disease worldwide. Low temperature and high relative humidity are factors suitable to the wide distribution of the disease (Stubbs, 1988; Johnson, 1988 and Danial, 1994). In Egypt, yellow rust is a sporadic disease because it appears in same year in Near and Middle East regions. However, starting from 1990s, it became common due to its continuous appearance (Abu El-Naga, 2001)

The disease was epidemic on several wheat varieties including Giza 144 (at Manzala district in 1967/68), Sakha 69, Giza 163, Gemmeiza 1 and most of the commercial varieties especially the long spiked ones at the Northern governorates in 1995/96 and 1997/98 growing seasons (Abdel-Hak, *et al*., 1972; El-Daoudi, *et al*., 1996 and Abu El-Naga, *et al*., 1999, 2001). Currently, more than 30 *Yr* genes have been identified and characterized worldwide**.** In Egypt, studies have shown that *Yr1, Yr3, Yr4, Yr5, Yr10, Yr15 ,YrSP* genes are still resistant against yellow rust disease while *Yr2, Yr6, Yr7* and *Yr9* genes have lost their efficacy (Shahin *et al*., 2011). The objective of this paper was to identify physiological races of Pst and their virulence on wheat in Egypt during 2009/2010 and 2010/2011 growing seasons.

MATERIALS AND METHODS

Field surveys for races of yellow rust pathogen. Regular field surveys were conducted across wheat growing areas in Northern governorates of Egypt *i.e*. Kafr-Elsheikh, Dakahliya and Gharbiya, in addition to the field of wheat breeding program. Infected wheat leaves were collected from trap nurseries and the commercial wheat fields. Data of location, cultivar, disease severity, collector and any other relevant information were recorded for each sample.

The collected samples were purified using the single pustule technique and multiplied on one or two of the following susceptible checks i.e. *Triticum spelta saharensis*, Morocco and Giza 160. Seedlings of 10 days old of the above mentioned entries were atomized with sterile distilled water, gently rubbed between fingers in the presence of water mixed with few droplets of an adhesive material such as Tween 20, to remove the waxy layer on leaf in order to uphold more uredospores on the leaf blade following the methods adopted by Stubbes (1988) in which uredospores were suspended in mineral oil or nonphytotoxic paraffinic oil. The spores were multiplied on susceptible wheat cultivar to produce enough spores. A little piece of the sample yellow rusted leaf was placed in Petri's plates (10-cm diameter) containing filter paper moistened with sterile distilled water to induce sporulation of the pathogen and then the plates were kept in a fridge, at 4°c until they were inoculated on differential sets.

Inoculated seedlings were kept in a humid chamber for 24-48h in darkness at 10°C. then, transferred to the permanent cabinets at diurnal light regime at 15°C, light intensity 7500 lux and at least 95% relative humidity. The night conditions were 15°C, darkness, and >95% relative humidity. The day/night rhythm was 8/16h. These schemes were precisely applied as reported by Stubbs (1988).

Race identification: The differential wheat genotypes listed in Table (1) were planted in a climate room under the same temperature and light conditions described above. Infection types (ITs) data of the plant-pathogen interactions based on the 0 to 9 scale adopted by McNeal *et al*. (1971) were recorded 15-20 days after disease symptoms of Pst were developed.

Identification of stripe rust physiologic races was performed using the world and European group of wheat differential genotypes listed in Table (1), which were used according to the method of Johnson *et al*. (1972)**.** A number of sets of 7-10 day-old seedlings were inoculated, incubated and allowed to continue their growth until symptoms onset (ca 18-20 days). Disease records were estimated using the 0 to 9 scale adopted by McNeal *et al.* (1971) in which (0-5) are considered resistant responses, while (6-9) are susceptible responses. Race nomenclature was done based on their virulence according to the following equation:

$$\text{Virulence}(\%) = [\text{ Effective / ineffective host genes }] \times 100$$

Such equation was applied according to the method adopted by Green (1965).

Table (1). Differential wheat genotypes used for the identification of races of the stripe rust fungus (*Puccinia striiformis* f. sp. *tritici*) in Egypt.

Differential genotypes	Abbreviation	Decanery value	Resistance gene	Type
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	*Yr SD*	Winter
Suwon 92 × Omar	Su	(2^6) = 64	*Yr SU*	Winter
Clement	Cl	(2^7) = 128	*Yr2* ,*Yr9*$^+$	Winter
Tirticum spelta album	Sp	(2^8) = 256	*Yr5*	Spring
European Differential set				
Hybrid 46	H46	(2^0) =1	*Yr 4*	Winter
Reichersberg 42	R42	(2^1) = 2	*Yr7*$^+$	Winter
Heines Peko	Pe	(2^2) = 4	*Yr2, Yr6*$^+$	Spring
Nord Desprez	No	(2^3) = 8	*Yr3*$^+$	Winter
Compare	Com	(2^4) = 16	*Yr8*	Spring
Carstens V	CV	(2^5) = 32	*Yr23*$^+$	Winter
Spaldings Prolific	Spa	(2^6) = 64	*YrSP*	Winter
Heines VII	HVII	(2^7) = 128	*Yr2*	Winter

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

Determination of gene efficacy: To evaluate stripe rust resistant genes, times of resistant reactions for every monogenic line were recorded as a percentage of the total number of isolates following Green (1965

Gene efficacy=No. of avirulent isolates/ Total number of isolates × 100

RESULTS

Identification of physiological races:

a. The first season 2009/10:

Data presented in Table (2) revealed the occurrence of seven races of stripe rust (Pst). These races were 0E0, 4E0, 16E2, 16E128, 16E130, 60E153 and 60E177, which were determined based on sum of high infection types for each of 17 wheat stripe rust monogenic differentials.

Table (2).Wheat yellow rust pathotype identity, their frequency and virulence in Egypt during 2009/2010 growing season.

No.	Races	No. of pathotypes	Frequency (%)	Virulence/avirulence*
1	0E0	10	33.33	/1,7,6,3,10,SD,SU,9,5,4,7^+,6^+,3^+,8,CV,SP,2
2	4E0	6	20.00	6/1,7,3,10,SD,SU,9,5,4,7^+,6^+,3^+,8,CV,SP,2
3	16E2	5	16.66	10,7^+/1,7,6,3,SD,SU,9,5,4,6^+,3^+,8,CV,SP,2
4	16E128	3	10.00	10,2/1,7,6,3, SD,SU,9,5,4,$7^+$$6^+$,$3^+$,8,CV,SP
5	16E130	3	10.00	10,7^+,2/1,7,6,3, SD,SU,9,5,4, 6^+,3^+,8,CV,SP
6	60E153	2	6.66	6,3,10,SD,4,3^+,8,2/1,7,SU,9,5,7^+,6^+,CV,SP
7	60E177	1	3.30	6,3,10,SD,4,8,CV,2/1,7,SU,9,5,7^+,6^+,3^+,SP

***Yellow rust resistance genes on the right side of the slash, and susceptible genes are on the left sides of the slash.**

The survey samples of the 2008/09 were collected from Northern governorates of Egypt. These data revealed that race 0E0 was the most frequent one (33.33%) followed by race 4E0 (20.00 %) and 16E2 (16.66%), while race 16E128 and race 16E130 were frequented at 10%. Races 60E153 and 60E177 were the least frequent ones (6.66 and 3.30%).

b. The second season 2010/11:

Data presented in Table (3) revealed the occurrence of 13 races of Pst. These races were 0E0, 6E0, 2E0, 2E16, 4E0, 4E4, 6E5, 6E20, 18E16, 34E16, 34E20, 38E20 and 70E4, which were determined based on sum of high infection types for each of 17 wheat stripe rust monogenic differentials.

Table (3). Wheat yellow rust pathotype identity, their frequency and virulence in Egypt during 2010/2011 growing season.

No.	Races	No. of Pathotypes	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,4 /1,3,10,SD,9,5,4,7^+,3^+,8,CV,SP,2

***Yellow rust resistance genes on the right side of the slash, and susceptible genes are on the left sides of the slash.**

The surveyed samples of the 2010/11 season in Northern governorates of Egypt are clarified in Table (3). These data revealed that race 6E4 was the most frequent one (16.66%) followed by race 70E40 (13.33%), race 38E20 (11.66%), race 4E4 (10%), and races 0E0 and 6E5

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

Frequency of virulence: Frequency of virulence of the causal organism Pst was studied using rust samples collected from North governorate of Egypt during 2008/2009 and 2009/2010 growing seasons. The obtained samples were tested in the following season 2009/2010, and 2010/2011. Virulence was tested against 17 monogenic lines for stripe rust resistance. Frequency of virulence was estimated as virulent isolates to the total number of isolates for each wheat genotype.

Frequency of virulence to *Yr's* genes:

Occurrence of virulence in 2009/10, and 2010/11: The results presented in Table (4) show varied frequencies of virulence to the tested lines. During 2009/10, the lowest values were recorded against *Yr1, Yr7, YrSU, Yr9, Yr5, Yr 6*$^{+}$ and *YrSP* (0 %). On the other hand, the highest occurrence of virulence was recorded with *Yr10, Yr 7*$^{+}$*, Yr2, Yr6, Yr3, Yr4* and *Yr8* ranging from 46.66 to 10% in a descending order. Whereas, the rest of lines showed moderate responses as shown in Table (4) during 2009/10.

Table (4). Stripe rust effective genes, times of resistant responses and efficacy action within physiological races in 2009/10 and 2010/11 growing seasons.

*Yr's**	2009/10 growing season				2010/11 growing season			
	No. of avirulent isolates	No. of virulent isolates	Virulent frequency %	Gene efficacy %	No. of avirulent isolates	No. of virulent isolates	Virulent frequency %	Gene efficacy %
1	30	0	0.00	100	60	0	0.00	100.00
7	30	0	0.00	100	17	43	71.66	28.33
6	21	9	30.00	70.00	14	46	76.66	23.33
3	27	3	10.00	90.00	60	0	0.00	100.00
10	16	14	46.66	53.33	58	2	3.33	96.66
SD	27	3	10.00	90.00	53	7	11.66	88.33
SU	30	0	0.00	100.00	51	9	15.00	85.00
9	30	0	0.00	100.00	60	0	0.00	100.00
5	30	0	0.00	100.00	60	0	0.00	100.00
4	27	3	10.00	90.00	55	5	8.33	91.66
(7)	22	8	26.66	73.33	60	0	0.00	100.00
(6)	30	0	0.00	100.00	20	40	66.66	33.33
(3)	28	2	6.66	93.33	60	0	0.00	100.00
8	27	3	10.00	90.00	43	17	28.33	71.66
CV	29	1	3.33	96.66	60	0	0.00	100.00
SP	30	0	0.00	100.00	60	0	0.00	100.00
2	21	9	30.00	70.00	60	0	0.00	100.00

Total isolates 30 (first season), 60 (second season) / *SD = Strubs Dickhof, SU = Suan × Omar, CV= Carsten V, SP = Splding prolific.

During 2010/11 growing season, the lowest frequencies of virulence were recorded with *Yr's* i.e. *Yr1, Yr7, YrSu, Yr9, Yr5, Yr 7*$^{+}$ and *YrSP*. On the other hand, *Yr6, Yr7, Yr 6*$^{+}$ and *Yr8* have yielded virulence frequency of 76.66, 71.66, 66.66 and 28.33 respectively.

DISCUSSION

Wheat stripe rust was known as a sporadic disease in Egypt during the elapsed decades. However, with the beginning of the 1990's, its occurrence seemed to be continuous every year (Abu El-Naga, 2001). The annual survey of wheat stripe rust conducted throughout two growing seasons of wheat crop in certain governorates of Egypt gave

The present work gave evidence to the presence of 7 physiological races of Pst during 2009/10, and 13 ones during 2010/11. Race 0E0 exhibited the highest frequency during the first season, while race 6E4 exhibited the highest frequency during the second season. Among all races, only 0E0 and 4E0 have occurred in both seasons. The rest of races appeared in either season. Similar results were reported by Abu El-Naga *et al.* (1999 and 2001), Ashmawy (2005) and Youssef *et al*. (2003, 2006 and 2007), who confirmed the predominance of races 0E0, 4E0, 2E16 and 70E40 in Egypt. The race 0E0 was also reported earlier at Algeria and Morocco during 1990-1992. This may be attributed to that the genes it attacks are not present in the differentials set. So, it must be supplemented with a complementary group of monogenics, preferably a local Egyptian group (Abu El-Naga, 2000; Shahin, 2008; and Shahin and Abu El-Naga, 2011). Race 2E0 was recorded at initial public offering (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 counties other than Egypt (Yahyaoui *et al*., 2002; Hakim and Yahyaoui, 2003; Wellings, 2007; and Ashmawy, 2010).

The obtained results indicated the good performance of Yr's 1, 9, and 5 and SP, since they could not be attacked all over the two seasons (100% efficacy) by any of the tested races. Likewise, Yr's CV, 3^+, 4 and 10 occupied the second rank *i.e.* (>90%). Yr's *SD, SU*, and 8 came in the third rank (>80%). While, Yr' 6, 7^+, and 6^+ were the least in this regard. These results were supported by those of Abu El-Naga *et al*. (1999 and 2001) and Youssef *et al*. (2003, 2006 and 2007).

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تعريف السلالات الفسيولوجية وعدوانية فطر الصدأ الأصفر علي القمح في مصر

ياسر محمد نور الدين شبانه[1] ، عاطف عبدالفتاح شاهين[2] و هند عبدالنبى عمر[2]

[1] قسم امراض النبات ، كلية الزراعة ، جامعة المنصورة ، مصر

[2] معهد بحوث أمراض النباتات ، قسم أمراض القمح ، مركز البحوث الزراعية ، مصر

يعتبر مرض الصدأ الأصفر من أهم الأمراض التى تصيب القمح فى مصر وعلى مستوى العالم. تهدف الدراسة إلى تحديد الجينات الفعالة في مقاومة المرض بهدف إدخالها فى برنامج تربية القمح لإنتاج أصناف مقاومة عالية الإنتاج. وفى هذه الدراسة تم تعريف سبعة سلالات فسيولوجية من الفطر المسبب للمرض (بكسينيا ستراي فورميس) فى الموسم 2010/2009 وكانت هذه السلالات هى: 0E0, 4E0, 16E2, 16E128, 16E130, 60E153, 60E177. ، أما فى موسم 2011/2010 تم تعريف ثلاث عشرة سلالة فسيولوجية وهى: 0E0, 6E0, 6E4, 2E0, 2E16, 4E0, 4E4, 6E5, 6E20, 18E16, 34E20, 38E20, 70E4. وكانت السلاله 0E0 هى الأكثر تكراراً يليها السلاله 4E0 و6E4 خلال موسمى الدراسة. وكانت الجينات Yr's 1,5,9,SU,SP هى الأكثر فاعلية في مقاومة المرض ، بينما كانت الجينات Yr's 7,6,6+ هى الأقل فاعلية خلال موسمى الدراسة تحت الظروف المصرية.

قام بتحكيم البحث

أ.د / محمد السيد عبد اللة	**كلية الزراعة – جامعة المنصورة**
أ.د / واصف عبد الصمد يوسف	**مركز البحوث الزراعية**