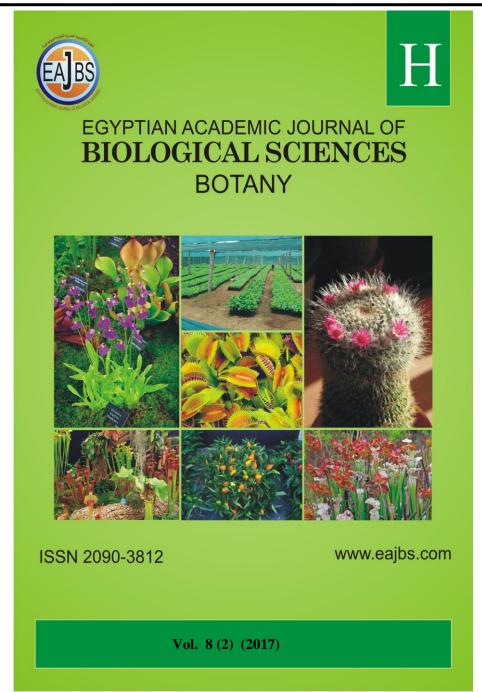
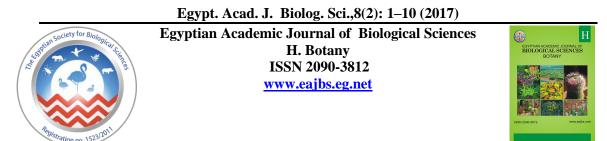
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Effective Genes for Resistance to Wheat Yellow Rust and Virulence of Puccinia striiformis f. sp. tritici in Egypt

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ABSTRACT

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Yellow (stripe) rust of wheat (Triticum aestivum L.), caused by Puccinia striiformis f. sp. tritici, is a serious problem of wheat production in many parts of the world including Egypt. Virulence patterns of wheat stripe rust were studied under the field conditions in four growing seasons (2013 to 2017) at Sakha Agriculture Research Station by planting international stripe rust trap nursery of differentials sets, isogenic lines and some Egyptian genotypes. The results revealed that stripe rust resistance genes Yr2+, Yr5, Yr10, Yr15 and YrND were effective, while Yr18 and Yr29 were partially effective during study periods showing moderate susceptibility. Genes Yr2, Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr32, and gene combinations Yr27+Yr18 (Opata 85) were found susceptible. Chinese 166 with resistance gene Yr1 has shown susceptible reaction in 2014/2015 growing season. Among the twenty commercial genotypes; Misr 1, Misr 3, Gemmeiza 7, and Sakha 95 were resistant. On other hand, some of the most important commercial genotypes *i.e.* Sids 12, Giza 168, Misr 2 and Sakha 61, known as resistant to the previously characterized races of yellow rust in Egypt became susceptible in this study. The genes found effective against yellow rust under natural conditions could be deployed singly or in combination to develop yellow stripe resistant and high yielding wheat varieties in Egypt.

INTRODUCTION

Yellow (stripe) rust caused by *Puccinia striiformis* Westend f. sp. *tritici* Eriks, is the major foliar disease of wheat, resulting in yield loss all over the world (Kolmer, 1966). Stripe rust severely damages wheat production worldwide (Roelf *et al.*, 1992; Line, 2002) causing yield losses from 10 to 70% besides affecting the quality of grain and forage (Chen, 2005). In Egypt, four sudden disease epidemics were recorded during the five elapsed decades (1967 - 1997). The first yellow rust epidemic was recorded in 1967; however, the most important stripe rust epidemic occurred in 1995 particularly in the Northern and Southern Delta areas. Yield of the most common commercial wheat varieties were significantly reduced by yellow rust and the national average loss in grain yield ranged from 14.00 - 20.50 % in the Nile delta region (El-Daoudi *et al.*, 1996). Screening of varieties against stripe rust is a

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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., 2002a). First study on specialization and changes in virulence factors of wheat yellow rust have been conducted by trap nursery to study annual changes of races and virulence factors of wheat yellow rust in Europe and the spread of races over long distances was reported by (Zadoks, 1961). The specific interactions between host resistance genes and pathogen avirulence genes serve as useful markers for characterizing rust populations. Near-isogenic or single-gene wheat lines are used to identify virulence patterns of rust pathogens (Samborski and Dyck, 1976). This helps in devising and managing crops through selecting effective resistance in high yielding wheat varieties. The objective of the study was to evaluate reaction of the differential set, isogenic line and Egyptian genotypes to identify the virulence of yellow rust pathogen and determine the effectiveness of resistance genes since 2013-2017.

MATERIALS AND METHODS

Plant materials and field experiments: The 47 differential sets and isogenic lines were received from ICARDA with known yellow rust resistance genes and 20 spring wheat entries representing the Egyptian wheat germplasm (*T. aestivum* L.) were chosen for this experiment received from Wheat Research Department, Field Crops Institute, Agricultural Research Center (ARC), Egypt as listed in Table (1).

Table 1. List of the tested Egyptian wheat genotypes, and their pedigree.

Ν	Genotypes	Padiana	Voor of voloogo
0.		Pedigree	Year of release
1	Misr 1	CMSS00Y01881T-050M-030Y-030M-030WGY-33M-0Y-0S.	2010
2	Misr 2	CMSS96M03611S-M-010SY-010M-010SY-8M-0Y-0S.	2011
3	Misr 3	CMSS06Y00582T099TOPM-099Y-099ZTM-009Y-099M-10WGY-0B-0EGY.	-
4	Sakha 8	PK3418-6S-0S-0S.	1978
5	Sakha 61	CM15430-2S-6S-0S-0S.	1980
6	Sakha 93	S.8871-1S-2S-1S-0S.	1999
7	Sakha 94	CMBW90Y3180-0TOPM-3Y-010M-010M-010Y-10M-015Y-0Y-0AP-0S.	2004
8	Sakha 95	CMA01Y00158S-040POY-040M-030ZTM-040SY-26M-0Y-0SY-0S.	-
9	Gemmeiza 3	BB/7C*2//Y50/KAL*3//Sakha8/4/PRV/WW/5/BJI/''S''//ON*3/BON.	1995
10	Gemmeiza 7	GM4611-2GM-3GM-1GM-0GM.	2000
11	Gemmeiza 9	GM 4583-5GM-1GM-0GM.	2000
12	Gemmeiza 10	CG5820-3GM-1GM-2GM-0GM.	2004
13	Gemmeiza 11	GM7892-2GM-1GM-2GM-1GM-0GM.	-
14	Gemmeiza 12	CMSS97Y00227S-5Y-010M-010Y-010M-2Y-1M-0Y-0GM.	-
15	Giza 168	CM93046-8M-0Y-0M-2Y-0B-0SH.	1999
16	Giza 171	S.6-1GZ-4GZ-1GZ-2GZ-0S	-
17	Sids 9	SD10002-14SD-3SD-1SD-0SD.	
18	Sids 12	SD7096-4SD-1SD-1SD-0SD.	2007
19	Sids 13	ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP-0SD.	2010
20	Shandaweel 1	CMSS93B00567S-72Y-010M-010Y-010M-3Y-0M-0HTY-0SH.	2011

This experiment was conducted under natural conditions at Sakha Agricultural Research Station, in Kafrelsheikh during four years from 2013 to 2017. The differential sets and isogenic lines were used to identify virulence or avirulence for the current populations of stripe rust pathogen in present study. Each entry was planted in two 1 m rows, which were spaced 30 cm apart. Susceptible check (Morocco) row was planted around the border of the experiment with 10 entry intervals. The twenty Egyptian germplasm including the susceptible check (Morocco) were planted in Randomized Complete Block Design (RCBD) with three replications during crops-growing seasons 2013-2017. All the recommended cultural practices were carried out during the experiment.

Disease assessment

Rust severity was recorded on flag leaves after flowering when infection on cv. Morocco reached at least 50% severity. Severity estimates were based on the modified Cobb's scale (Peterson *et al.*, 1948), and reaction based on (Roelfs *et al.*, 1992). Coefficient of infection (CI) were calculated by combinations of disease severity (DS) and infection type (IT), was used for estimating Area Under Disease Progress Curve (AUDPC). Constant values for infection types were used since (immune =0, R= 0.2, MR=0.4, MR-MS=0.6, MS=0.8, S=1; Stubbs *et al.*, (1986). Estimation of AUDPC and rAUDPC was performed according to the following equation adopted by (Milus and Line, 1986).

AUDPC = D [$\frac{1}{2}(Y_1 + Y_k) + Y_2 + Y_3 + \dots + Y_{k-1}].$

Where, D = Time interval (days between reading), (Y_1+Y_k) = Sum of first and last disease scores. $(Y_2+Y_3 + ... + Y_{k-1})$ = Sum of all in-between disease scores.

rAUDPC = line AUDPC / susceptible AUDPC \times 100.

Then, they obtained data were statistically analyzed by MSTAT-C program and by using MS-Excel program as well for correlation analysis.

RESULTS

The differential sets and isogenic lines showed wide range of rust response during the four-year-investigation. The field data obtained in four cropping seasons revealed that resistance genes, Yr2+, Yr5, Yr10, Yr15, YrSD, YrND and were effective Table (2). The genotypes with Yr2, Yr6, Yr7, Yr9, Yr17, Yr27, YrSU, and YrA were susceptible. The genotype with Yr29 was moderately resistant while that with Yr18 with was moderately susceptible and Anza (YrA+Yr18) were moderately susceptible. The genotypes with Yr4, Yr5, Yr10, and Yr15, showed 0-type or R-type reaction suggesting these genes to be immune or resistant against the pathogen population's at four growing seasons. Sakha Station considered to be stripe rust hot spot in Egypt. In the previous study, no virulence was observed on plants with genes Yr1 (Chinese 166), (Spalding Prolific) YrSP and Yr32 in trap nursery, but in this study occurrence of virulence on previous mentioned genes in 2015/2016 which showed change of the pathogen populations. In addition to, the genotype responses were compared across the four seasons; the responses at 2015/2016 were more different, while those at three other growing seasons were relatively similar.

Based on the reaction of the isogenic lines, the yellow rust populations in the four seasons (2013 to 2017) were virulent to YrA, Yr2, Yr6, Yr7, Yr8, Yr9, Yr17, and Yr27. Avirulence to Yr2+, Yr5, Yr10, Yr15, and Yr26 while virulence to Yr1 was detected in 2015/2016, and no virulence was recorded at three other growing seasons. Partial virulence was recorded at four seasons for YrA, Yr18 (Anza).

	seventy an	u 115 produ	Severity and IT ^b of genotypes to stripe rust					
No.	Genotypes	Yr Gene/s ^a	2013/014	2016/17				
1	Avocet 'S'	-	60S	2014/15 50S	2015/16 30S	405		
2	Avocet 'R'	YrA	60S	50S	205	30S		
3	Yr1/6*Avocet S	Yr1	0	50B 5MR	5MR	0		
4	Yr5/6*Avocet S	Yr5	0	0	0	0		
5	Yr6/6*Avocet S	Yr6	60S	60S	40S	60S		
6	Yr7/6*Avocet S	Yr7	50S	50S	305	50S		
7	Yr8/6*Avocet S	Yr8	50S	20MS	40MRMS	10MSS		
8	Yr9/6*Avocet S	Yr9	20S	80S	305	50S		
9	Yr10/6*Avocet S	Yr10	TR	0	0	0		
10	Yr15/6*Avocet S	Yr15	0	0	0	0		
11	Yr17/6*Avocet S	Yr17	5MR	10S	0	10MS		
12	Yr18/6*Avocet S	Yr18	20MS	20MS	5MS	5MSS		
13	Yr27/6*Avocet S	Yr27	5MS	30MSS	20MS	10MSS		
14	<i>Yr32/6</i> *Avocet S	Yr32	0	30MSS	0	5S		
15	YrSP/6*Avocet S	YrSP	0	5R	5R	0		
16	Chinese 166	Yr1	0	0	10S	0		
17	Lee	Yr7	205	205	10S	205		
18	Heine's Kolben	Yr6+1	20S	10MS	10MRMS	10MSS		
19	Vilmorin 23	Yr3a, 4a + ?	0	10MS	0	5R		
20	Moro	Yr10	0	0	0	0		
20 21	Strubes Dickopf	YrSD	0	5R	0	TR		
21 22	Suwon 92/Omar	YrSU	5R	0 0	TS	TS		
22	Clement	Yr9, Yr2+?	5R	5MR	0	5MR		
23 24	Hybrid 46	Yr4	0 0	0	0	0		
24 25		Yr7+?		10MR	0	0		
23 26	Reichersberg 42	Yr6+?	0 0	0	5MS	5R		
20 27	Heine's Peko	YrND	0	5R				
27	Nord Desprez		TMR	5MRMS	0 TMR	0 5145		
	Compare	Yr8				5MS		
29 30	Carstens V	Yr32	0 TR	10MRMS	0 TC	TR		
	Spalding Prolific	YrSP		5R	TS	5S		
31 32	Heines VII	Yr2+?	0 5R	5R 5R	0 TS	TR TS		
	Spaldings Prolific	YrSP V::0			1 S 10MRMS			
34 25	Federation4/Kavkaz	Yr9 V:: 4 V:: 18	10MRMS	20MSS		10MS		
35	Anza	YrA, Yr18	5MR	5MRMS	5MS	10MS		
36	Kalyansona	Yr2 V:-5	5MR	20MS	10MS	20MSS		
37	Triticum spelta album	Yr5	0	5MR	0	0		
38	TP1295	Yr25	30S	30S	10MSS	20S		
39	Jupateco 'R'	Yr18+	5MRMS	20MS	5MSS	5MS		
40	Fielder	Yr6, Yr20	40S	60S	30S	30S		
41	Lemhi	Yr21	30S	80S	20S	20S		
42	Lal Bahadur/Pavon 1BL	Yr29	5MR-MS	5MS	5MS	TMS		
43	Opata 85	Yr27, Yr18	10MS	30MRMS	10S	10MSS		
44	Ciano 79	<i>Yr27</i>	20MS	10MSS	20S	20MSS		
45	Yr28/Avocet	Yr28	30S	40S	20S	30S		
46	Pavon 76	Yr29, Yr30	TMRMS	5MSS	5MS	10MRMS		
47	Pastor	Yr31, APR	10MS	5MSS	5MS	5MS		
48	Morocco Resistance genes based on the stud	-	70S	$\frac{80S}{1000}$	100S	80S		

Table 2. Wheat genotypes used in trap nursery, their resistance genes, severity and ITs produced by yellow rust during 2013–2017.

¹/05 <u>605</u> <u>1005</u> <u>805</u> ^aResistance genes based on the studies of (Chen, 2005)., ^bITs based on (Roelfs *et al.*, 1992)., 0=Immune. R=Resistant without sporulation. TMR=Trace moderately resistant. MR=moderately resistant; small pustules surrounded by necrotic by necrotic areas. MS=moderately susceptible; medium-sized pustules, no necrosis, but some chlorosis possible. MSS=moderately susceptible to susceptible; medium- to large-sized pustules without chlorosis or necrosis. S=susceptible; large pustules, no necrosis or chlorosis.

Data in Tables (3 and 4) show the evaluation of 20 entries at the adult stage during four seasons. The Egyptian wheat genotypes had more variation in reaction through the four growing seasons, as observed for genotypes and years for FRS, AUDPC, and final ACI. The FRS of susceptible control, Morocco was 70 to 100%,

indicating that high adult plant infection type was established over four-year field experiment. The genotypes Misr 3, and Sakha 95 that have uncharacterized genes were resistant, one variety (Gemmeiza 7) showed moderate resistance, three varieties (Gemm. 3, 10 and 12) were moderately susceptible and the remaining varieties were susceptible.

The mean rust severity during 2015/16 was the highest across years. Twenty (20) varieties that had varied reactions may be classified into three classes. Class 1 included fifteen varieties (Misr 2, Gemmeiza 3, 9, 10, 11, 12, Sids 1, 12, 13, Sakha 8, 61, 69, 94, Giza 168 and Giza 171) showing susceptible to moderately susceptible reaction. Class 2 included, moderately resistant to resistant varieties (Misr 1 and Shandaweel 1) were the least affected during the four years, relative values for AUDPC and ACI were also the least for these two entries and were the most resistant. Varieties in class 3 included (Misr 3 and Sakha 95), showing R-type or 0-type reaction. Most Egyptian varieties were susceptible and their susceptibility levels were higher at the 2015 growing seasons than in the other seasons of experimentation under field condition at Sakha during 2014-2017.

Table 3. The response of 20 wheat entries against stripe rust infection under field condition at Sakha during 2013/2017.

No.	Genotypes	2013/014	2014/15	2015/16	2016/17
1	Misr 1	0	5R	5R-MR	5MR
2	Misr 2	TMR	5MR	5S	5S
3	Misr 3	-	-	0	0
4	Gemmeiza 3	5MS	5MSS	5S	5 MRMS
5	Gemmeiza 7	TMR	TMR	5MR	10MRMS
6	Gemmeiza 9	5MSS	10MR-MS	20MS	10MSS
7	Gemmeiza 10	0	10MR	5MS	10MS
8	Gemmeiza 11	0	10 MS	20S	10S
9	Gemmeiza 12	-	-	10MRMS	5MS
10	Sids 1	5MR-MR	5MS	10MSS	10S
11	Sids 12	20MSS	20S	50S	40S
12	Sids 13	5MS	20MSS	30S	20S
13	Sakha 8	20S	10S	10S	10S
14	Sakha 61	0	0	5S	10S
15	Sakha 69	40S	30S	30S	20S
16	Sakha 94	10MR	20MS	20MSS	10MSS
17	Sakha 95	-	-	0	0
18	Giza 168	5MR	5MS	20S	20S
19	Giza 171	-	-	5S	TrS
20	Shandaweel 1	0	10MR	10MRMS	5MRMS
21	Morocco	70S	80S	100S	80S

Among the Egyptian varieties, Gemmeiza 9 and Misr 2 showed partial virulence at 2013/14 Season, while complete virulence was recorded at 2016/17 Season. Furthermore, differences in virulence pattern were observed in consecutive years, especially variety, Sakha 61 showed immunity in 2013/14 and 2014/2015 while complete virulence was recorded at 2016/17. Based on rAUDPC values, data indicated that cvs. Misr 1, and Shandweel 1 were the least values, while the highest value was recorded with Sids 12 and Sids 13. With regard to the mean of AUDPC and coefficient of infection the least area of disease progression were observed with Misr 1 while that the highest values recorded with Sids 12 Table (4) and Fig (1).

Table 4. Adult plant infection type and mean comparison for coefficient of infection, AUDPC and rAUDPC in 20 Egyptian wheat genotypes against stripe rust.

			Mean coefficient of	Mean	
No.	Genotypes	FRS	infection	AUDPC	Mean of r AUDPC
1	Misr 1	MR	3.25 ^e	36.25 ^r	1.86 ^t
2	Misr 2	S	5.23 ^s	97.89^{1}	4.55 ¹
3	Misr 3	0	0.00^{t}	0.00 ^s	0.00 ^u
4	Gemmeiza 3	MRMS	10.75 ^k	63.75 ⁿ	2.96 ^q
5	Gemmeiza 7	MRMS	$7.50^{ m q}$	56.00 ^{op}	2.60 ^r
6	Gemmeiza 9	MSS	9.88 ^m	116.25 ^k	5.40 ^k
7	Gemmeiza 10	MS	8.50 ⁿ	72.50 ^m	3.37 ^{op}
8	Gemmeiza 11	S	20.75 ^f	167.50 ⁱ	7.78 ⁱ
9	Gemmeiza 12	MS	7.23 ^r	97.50^{1}	4.53 ^m
10	Sids 1	S	18.50 ⁱ	325.75 ^e	15.14 ^e
11	Sids 12	S	40.50 ^b	815.00 ^b	37.89 ^b
12	Sids 13	S	27.50 ^d	650.00 ^c	30.22 ^c
13	Sakha 8	S	20.00 ^h	268.75 ^g	12.49 ^g
14	Sakha 61	MSS	10.75 ^k	95.75 ¹	4.45 ⁿ
15	Sakha 69	S	30.00 ^c	637.5 ^d	29.64 ^d
16	Sakha 94	MSS	7.63 ^{op}	195.25 ^h	9.07 ^h
17	Sakha 95	0	0.00^{t}	0.00s	0.00 ^u
18	Giza 168	S	20.25 ^g	275.63^{f}	12.81 ^f
19	Giza 171	MSS	10.13 ¹	125.63 ^j	5.84 ^j
20	Shandaweel 1	MRMS	10.80 ^j	50.25 ^q	2.33 ^s
21	Morocco	S	97.50ª	2150.50 ^a	100.00 ^a
LSD	1%	0.01	0.006	6.957	0.002
	5%	0.05	0.007	5.203	0.001

*Means followed by the same letters in each column are not statistically different at 1% level.

Association between slow rusting parameters:

Field assessment of slow rusting resistance was evaluated through CI and rAUDPC. CI is the mostly used parameter for the purpose. During in this study, an attempt was made to elucidate the relationship between these parameters. Positive relation of coefficient of infection was found with and rAUDPC with a strong R^2 value that was 96% Fig. (2).

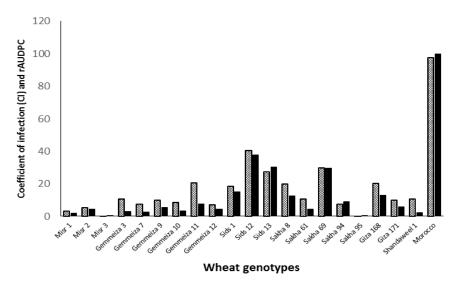


Fig. 1. Average coefficient of infection (CI) and rAUDPC of twenty genotypes evaluated under natural infection of stripe rust at Sakha in 2017 growing season.

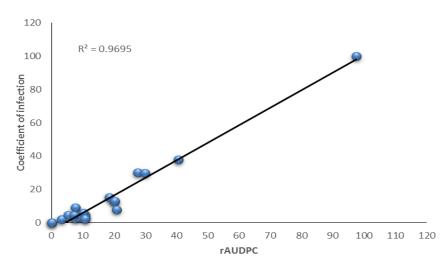


Fig. 2. Association between coefficient of infection (CI) and rAUDPC for assessment of genotypes.

DISCUSSION

The genotypes received from ICARDA and selected Egyptian genotypes received were used to identify virulence, and avirulence for the current populations of the stripe rust pathogen in various growing seasons of Egypt (from 2013 to 2017. The obtained results indicated the good performance of Yr's: 2+, 4, 5, 10, 15 and SD, as they could not be attacked all over the four growing seasons were effective against the yellow rust populations. Likewise Yr1, YrCV and YrSP occupied the second rank. In some countries, Chinese 166 with resistance gene Yrl has showed susceptible reaction in some years (Yahyaoui, 2006). Virulence for Yrl in some provinces of Egypt has also been found (Shahin, 2008) Yr's 2, 6, 7, and 9, came in the third rank. Yr18 which is an adult plant resistance gene, provided some level of resistance (and therefore, moderately susceptible) under the field conditions. Genes such as Yr5 are previously known to show high level of 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, virulence to Yr5 and Yr15 genes rarely occur in wheat producing areas of the world (Chen, 2005). In Egypt it must be mentioned herein that Yr5 was attacked by race 494E158 in 2006/2007; (Shahin, 2008; Shahin and Abu El-Naga, 2011). Building breeding programms major genes pose vulnerability. In contrast, minor genes that work in combination with other (major and/or minor) genes provide durable resistance (Bux et al., 2011). Durable resistance for stripe rust in many wheat varieties around the world has been attributed to the presence of Yr18 (Singh, 1992) and non-race-specific resistance genes (Chen, 2005).

Recent studies at CIMMYT (Singh *et al.*, 2005) have shown that the gene *Yr46* is closely linked to gene *Yr9*. Gene *Yr46* is also closely linked to *Lr67* (Herrera-Foessel *et al.*, 2011). These genes confer slow rusting to yellow and leaf rust. Another minor gene, *Yr30*, involved in the adult plant resistance of several CIMMYT wheat was found to be in the chromosomal region carrying durable stem rust resistance gene *Sr2* (Singh *et al.*, 2000). For durable resistance in wheat, breeding programs in Egypt should focus on the use of genes like *Yr18*, *Yr29*, *Yr30*, *Yr36* and *Yr39* and many other QTLs for adult-plant resistance or high-temperature adult-plant (HTAP) resistance (Chen, 2005). Concerning the evaluation of 20 local germplasm at adult stages against yellow rust under field conditions, however, their resistance genes are

unknown. The obtained results indicated that 4 entries out of 20 showed resistance through four seasons against stripe rust Misr 1, Misr 3, Sakha 95 and Shandweel 1. It could be concluded that these entries may exhibit durable resistance, in which it could be kept for long time irrespective off the change in pathogen genotype. One of the possibilities is that entries carry major genes as in resistant tester lines Yr5, Yr10, Yr15, and YrSP. However, further characterization is required especially through molecular marker using the gene postulation approach and generic analysis. The cultivars Gemmeiza 7 and Shandweel 1 were moderately resistant to moderately susceptible at adult plant stage. These cvs. which had low rAUDPC at adult stage could have durable resistance. Since all disease parameters (FRS, ACI, AUDPC, and r AUDPC) strongly and positively are most appropriate parameters for detecting slow rusting materials from a large number of breeding populations/lines. Varieties identified with slow rusting characteristic should be improved/developed further by accumulating 4-5 slow rusting genes to achieve near immunity (Singh et al., 2000b). The results of this study will assist in devising a strategy for yellow rust management, using the well-characterised wheat germplasm carrying effective resistance genes in the breeding programmes in Egypt.

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ARABIC SUMMARY

دراسة عدوانية فطر الصدأ الاصفر على القمح والجينات الفعالة تحت الظروف المصرية

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