Frequency of Virulence and Virulence Formula of Wheat Leaf Rust Races Identified in Egypt during 2004/05 - 2007/08

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heat leaf rust is one of the major diseases on wheat in Egypt which appears annually in virulence of different frequencies. In this investigation, leaf rust samples were collected from different locations of Lower Egypt during four seasons (2003/04–2006/07). The single pustule method of isolation was followed for each sample. Rust data were recorded as infection types and virulence frequencies were determined against 20 Lr genes, in monogenic lines, and some Egyptian genotypes. Virulence frequencies were very high against Lr's 1, 2a, 2c, 3, 16, 26, 11, 30, 2b, 14b, 15 and 42, but were the lowest against Lr's 9, 24, 3ka, 10, 12, 18, 21 and 36 and also the cv. Gemmeiza 9. The physiologic races were identified according to their reactions on the 20 Lr's and virulence formula (virulence/avirulence) was recorded for each race. Shift, appearance and disappearance of the physiologic races were demonstrated. Race groups TT---, PT--- and FT--- were the most dominant being virulent mainly against Lr's 1, 2c, 3 and 9.

Keywords: Isogenic lines, leaf rust, *Puccinia triticina*, race groups, wheat, virulence frequency.

Leaf rust (Puccinia triticina Erikss.= P. recondita f.sp. tritici) is a common disease on wheat in all growing areas in Egypt. It annually appears at varying magnitudes in the different locations of the country. Nevertheless, it occurs at higher levels of disease severities in Lower Egypt than the other parts of the country (Abdel-Hak et al., 1966 and Nazim et al., 1976).

Generally, disease infection and development depend on the host response, pathogen virulence and the prevailing environmental conditions (Dyck and Johnston, 1983 and Eversmeyer and Kramer, 2000).

Under the Egyptian conditions, almost the local wheat genotypes are susceptible to leaf rust in terms of infection types (Nazim et al., 1984). Moreover, the prevailing environmental conditions are highly suitable to the disease infection and development. Therefore, intensive care should be given to the prevailing virulences of the causal organism. However, it should be mentioned that the over summering of the causal organism urediniospores is doubtful and the main or the only source of inocula is from outside the country (Abdel-Hak et al., 1966 and Saari, 1976), particularly in the absence of the alternate host (Thalictrum spp.), volunteer plants

and/or stubs (Melchers, 1932 and Abdel-Hak et al., 1966). The urediniospores which come into the country are usually found in different pathotypes which differ in their virulence and aggressiveness against the known genes for leaf rust resistance and also against the local genotypes which are different in their genetic makeup (Nazim et al., 1983 and Nazim et al., 1984).

Therefore, the aim of this investigation was to study the frequency of virulence against the leaf rust single genes for resistance and also against the local genotypes, to serve the national breeding programme for resistance.

Materials and Methods

Infected leaf specimens were collected from different wheat growing areas in Egypt during 2003/04-2006/07. Samples were obtained from the commercial fields as well as from wheat leaf rust trap nursery (WLRTN), from lines carrying known genes for leaf rust resistance (Lr). Each specimen was transferred onto 8-9 day-old seedlings of the highly susceptible variety Giza 139 under greenhouse conditions.

Results

Frequency of virulence:

Frequency of virulence of the causal organism "Puccinia triticina Erikss." of wheat leaf rust was studied using rust samples collected from lower Egypt during 2003/04 - 2006/07. The obtained cultures were tested in the following seasons (2004/05 - 2007/08). Virulence was tested against 20 monogenic lines for leaf rust resistance (Table 1) and also against selected wheat local varieties. Occurrence of virulence was estimated as virulent isolates to the total number of isolates for each wheat genotype.

- a) Frequency of virulence to Lr's:
- 1) Occurrence of virulence in 2004/05:

The results presented in Table (3) showed different frequencies of virulence to the tested lines. The lowest values were found against Lr 9, Lr 21, Lr 24, Lr 10, Lr 36, and Lr 3ka, ranging from 19.58 to 36.08% in an ascending order. On the other hand, the highest occurrence of virulence was found against Lr 3, Lr 42, Lr 11, Lr 2a, Lr 15, Lr 30, Lr 16 and Lr 1, ranging 91.60 - 77.30% in a descending order. Whereas, the rest of lines showed moderate responses, as shown in Table (3).

2) Occurrence of virulence in 2005/06:

Results obtained revealed that Lr 9, Lr 21, Lr 36 and Lr 24 exhibited the lowest frequencies of virulence, being 25.0, 27.16, 27.71 and 36.14%, respectively. On the other hand, Lr 3, Lr 42, Lr 2c, Lr 15, Lr 30, Lr 16, Lr 14b, Lr 1, Lr 2b and Lr 26 arranged according to their virulence frequency, ranged from 93.01 to 73.8% (Table 3).

Table 1: "Pt-Code for the four differential sets in addition to a set of four lines for virulence survey for Puccinia triticina in Egypt

Infection t	vne b produced or	near isogenic lin	es (Lr lines):
1	29	2c	
1		24	26
		17	30
			2b
			42
14B	13		L
L	L		H
L	L	_	L
L	L		Н
L	L	n	L
L		- L	H
L		L	1
L	H		H
L	Н	Н	T T
Н	L	L	H
	L	L	n
	L		L
	L	H	H
	Н	L	L
		L	Н
		Н	L
		Н	H
	Infection to 1 1 9 3ka 10 14B L L L L L L L L H H H H H H H H H H H	Infection type b produced or 1	9 16 24 3ka 11 17 10 18 21 14B 15 36 L L L L L L L L H L L H L H L L H L H L L H L L H L L H L L H L L H L L H L L H L L H L L H L L H L L H L L H L L

^a Pt-code consists of the designation for set 1 followed by that for set 2, etc. For example, race MGBL; set I (M)-virulent to Lr 1, 3; set 2 (G)-virulent to Lr 16; set 3 (B)-avirulent; set 4 (L) virulent to Lr 10.

Table 2: Infection types of leaf rust of wheat

1105t respense	Infection * type	Disease symptom
(class)	type	No uredinia or other macroscopic sign of infection
[mmune	0	No uredinia or other macroscopic and No uredinia but hypersensitive necrotic or chlorotic
Nearly immune	0;	flecks present
Very resistant	1	Small uredinia surrounded by necrosis
Moderately resistant	2	Small to medium uredinia surrounded by necrosis Medium sized uredinia that may be associated with
Moderately susceptible	3	chlorosis
Very susceptible	4	Large uredinia without chlorosis
Heterogeneous	х	Random distribution of variable sized uredinia or single leaf

^{*} Infection types 0, 0; , 1 and 2 are considered as low infection types, whereas, infection types 3 and 4 are considered as high infection types (Johnston, 1961).

b L=low infection type (avirulent race); H = high infection type (virulent race).

^{*} Nazim (1998), W4 (1998-2001, W4, ATUT - Minufiya Univ., Egypt).

Table 3: Frequency of virulence of *Puccinia triticina* to 20 monogenic lines for leaf rust resistance during the following seasons

No.	Lr gene	Frequency of virulence (%) / season					
	ri gene	2004/05	2005/06	2006/07	2007/08		
1	Lr 1	77.30	75.00	65.50	89.20		
2	Lr 2a	85.56	55.90	74.78	79.68		
3	Lr 2c	85.40	89.10	76.40	81.50		
4	Lr 3	91.60	93.10	84.00	93.80		
5	Lr 9	19.58	25.00	25.21	23.07		
6	Lr 16	77.30	80.90	73.90	96.90		
7	Lr 24	31.95	36.14	27.73	30.96		
8	Lr 26	74.20	73.80	73.10	86.10		
9	Lr 3ka	36.08	40.47	31.09	35.38		
10	Lr 11	86.20	48.19	50.42	46.15		
11	Lr 17	46.87	40.24	68.90	53.84		
12	Lr 30	77.30	82.01	73.90	96.09		
13	<i>Lr</i> 10	32.97	37.03	42.37	44.16		
14	Lr 18	41.23	48.19	71.10	80.00		
15	Lr 21	25.77	27.16	28.81	21.53		
16	Lr 2b	75.70	75.90	80.50	41.50		
17	<i>Lr</i> 14b	73.10	77.30	84.00	98.40		
18	Lr 15	82.40	86.90	78.90	89.20		
19	Lr 36	36.00	27.71	30.25	32.30		
20	Lr 42	89.60	92.70	72.60	86.10		

3) Occurrence of virulence in 2006/07:

Virulence frequency against Lr 9, Lr 24, Lr 21, Lr 36 and Lr 3ka were the lowest, i.e. 25.21, 27.73, 28.81, 30.25 and 31.09%, respectively. While, virulence to Lr 1, Lr2a, Lr 2c, Lr 3, Lr 16, Lr 26, Lr 17, Lr 30, Lr 18, Lr 2b, Lr 14 b, Lr 15 and Lr 42 were the highest frequencies, being 65.5, 74.78, 76.4, 84.0, 73.9, 73.1, 68.9, 73.9, 71.1, 80.5, 84.0, 78.9 and 72.6%, respectively, (Table 3).

4) Occurrence of virulence in 2007/08:

The results given in Table (3) show different frequencies of virulence to the tested Lr genes in terms of infection types. The least frequencies were found to Lr 21, (21.53%) and Lr 9 (23.07%). On the other hand, 80% or more of the isolates were virulent to Lr 1, Lr 2 c, Lr 3, Lr 16, Lr 26, Lr 30, Lr 18, Lr 14 b, Lr 15 and Lr 42, while the rest of Lr genes showed different responses.

Dramatic shift in frequency of virulence to Lr 2a, Lr 16, Lr 11, Lr 17, Lr 18 and Lr 2 b was found, whereas Lr 1, Lr 2c, Lr 3, Lr 9, Lr 24, Lr 26, Lr 3 ka, Lr 30, Lr 10, Lr 21, Lr 14b, Lr 15, Lr 36 and Lr 42 were almost stable showing light shifts in their frequencies of virulence during the period of study. Moreover, no absolute resistance was noticed within the tested monogenic lines. The occurrence of virulences to Lr 9 and Lr 21 was the lowest, while, virulences to Lr 24 and Lr 36 were relatively moderate during the whole period of study.

b) Frequency of virulence to local wheat genotypes:

Occurrence of virulence was also estimated as percentage of virulent occurrence against the local wheat cultivars to the total number of the tested isolates.

1) Occurrence of virulence in 2004/05:

Data presented in Table (4) show that the lowest frequencies of virulence (18.50 and 29.33%) were found to wheat cultivars Gemmeiza 9 and Sakha 93, respectively. Relatively moderate frequencies (31.50, 31.90, 32.20, 37.14, 37.50, 37.90 and 47.90%) occurred to the wheat cultivars Sakha 94, Gemmeiza 10, Sids 10, Sakha 61, Gemmeiza 7, Sids 1 and Sids 9, respectively. Relatively higher frequencies occurred to other cultivars, *i.e.* Giza 164 (52.0%), Giza 170 (54.70%), Sakha 8 (55.60%), Sids 6 (57.20%), Sids 8 (61.40%) and Giza 168 (64.20%). Moreover, the highest frequencies (79.78, 72.16, 71.80 and 76.00%) occurred to the wheat cvs. Giza 160, Sakha 69, Sids 7 and Sids 11, respectively.

Table 4: Frequency of virulence of *Puccinia triticina* to Egyptian wheat varieties during the following seasons

No.	Variety	Fr	Frequency of virulence (%) / season				
1		2004 / 05	2005 / 06	2006/07	2007/08		
1	Giza 160	79.78	91.66	-	2007/00		
2	Giza 164	52.00	52.30	67.80	-		
3	Giza 168	64.20	67.80	72.00	20.30		
4	Giza 170	54.70	63.70	52.60	-		
5	Gem. 7	37.50	35.70	54.20	-		
6	Gem. 9	18.50	22.60	35.50	18.40		
.7	Gem. 10	31.90	33.30	43.20	7.60		
8	Sakha 8	55.60	60.70	68.60			
9	Sakha 61	37.14	84.50	79.80			
10	Sakha 69	72.16	84.50	88.90			
11	Sakha 93	29.33	88.00	75.40			
12	Sakha 94	31.50	47.40	65.20	9.20		
13	Sids 1	37.90	64.60	73.10	-		
14	Sids 6	57.20		-	-		
15	Sids 7	71.80	-	-			
16	Sids 8	61.40		-	-		
17.	Sids9	47.90		-	, - : -		
18	Sids 10	32.20		-	-		
19	Sids 11	76.00	75.00	71.50			
20	Sids 12	- 1	63.00	60.50	-		
21	Sohag 3		56.20	40.50	-		
22	Sohag l	-	35.30	24.30	_		
23	Baniswief 1		82.70	62.30			

2) Occurrence of virulence in 2005/06:

Data presented in Table (4) indicate that the lowest frequencies of virulence (22.6, 33.3, 35.7 and 35.3%) were detected to the wheat cvs. Gemmeiza 9, Gemmeiza 10, Gemmeiza 7 and Sohag 1, respectively. Higher frequencies occurred to the cvs. Giza 164, Giza 168, Giza 170, Sakha 8, Sakha 94, Sids 1, Sids 11, Sids 12 and Sohag 3, being 52.3, 67.8, 63.7, 60.7, 47.4, 64.6, 75.0, 63.0 and 52.2%, respectively. On the other hand, highest frequencies of virulence (91.66, 84.50, 84.50, 88.00 and 82.70%) were found to the wheat cvs. Giza 160, Sakha 61, Sakha 69, Sakha 93 and Baniswief 1, respectively.

3) Occurrence of virulence in 2006/07:

The lowest frequencies, *i.e.* 24.3, 35.5 and 43.2% were found to the wheat cvs. Baniswief 1, Gemmeiza 9 and Gemmeiza 10, respectively. Whereas, the highest frequencies (79.8, 88.9 and 75.4%) were found to the wheat cvs. Sakha 61, Sakha 69 and Sakha 93, respectively (Table 4).

4) Occurrence of virulence in 2007/08:

The results obtained in the fourth growing season showed that cvs. Gemmeiza 10 and Sakha 94 had low frequencies of virulence being, 7.6 and 9.2%, respectively, followed by cv. Gemmeiza 9 (18.4%) and cv. Giza 168 (20.3%).

From the results presented in Table (4), it was noticed that different virulence frequencies were found during the period of study. Occurrence of virulence to cvs. Gemmeiza 9 and Gemmeiza 10 was more stable, whereas, virulence to cvs. Giza 168 and Sakha 94 exhibited higher frequencies in the first three seasons, then declined in the fourth season.

Physiologic races and virulence formula:

Physiologic races of P. triticina were identified as major race groups showing different virulence formula (Table 5). Race group T- - - was the most dominant race group (40.0, 36.8, 27.4 and 19.9%), followed by race group P ---- (24.4, 13.7, 19.9 and 53.7%); race group F - - - (6.1, 4.7, 5.0 and 4.6%); race group B - - -(4.1, 4.7, 1.6%, -) and race group C - - - (3.0, 1.2 %, -, -,). Race groups TR - - and TP - - - occurred in lower percentages in the first three seasons and disappeared in the fourth season. On the other hand, race groups PL ---, PQ ---, PM --- and PP - - - , in addition to race group FQ - - - and BB - - - disappeared in the fourth season. Whereas, race group CT - - - disappeared in the last two seasons of the study. Moreover, race group TT - - - (27.8, 25.0, 22.6 & 18.4%) appeared in lower percentage of isolation in the fourth season, but race group PT - - - (12.3, 3.5, 11.7 & 47.6%) appeared in higher percentage in this season, reflecting shift in their occurrence in the fourth season of the study (2007 / 08). Moreover, virulence formula of the identified race groups, indicated that Lr's 2c, 3 and 9 were almost inactive against all identified races during the period of study (Table 5). Meanwhile, Lr's 1, 16, 24 and 26 were active against certain identified races and inactive against others.

Table 5: Virulence and Virulence formula of Puccinia triticina Erikss

Race group *		Isolation (%)/year**				
	Virulence formula	2004/05	2005/06	2006/07	2007/08	
TT	Lr 1,2a,2c,3,9,16,24,26	27.8	25.0	22.6	18.4	
TS	Lr 1,2a,2c,3,9,16,24	4.1	4.7	1.6	1.5	
TR	Lr 1,2a,2c,3,9,16,26	5.1	5.9	1.6	-	
TP	Lr 1,2a,2c,3,9,24,26	3.0	1.2	1.6	-	
T		40.0%	36.8%	27.4%	19.9%	
PT	Lr 1,2c,3;9,16,24,26	12.3	3.5	11.7	47.6	
PL	Lr 1,2c,3,9	1.0	13.0	0.8	-	
PS	Lr 1,2c,3,9,16,24	1.0	1.2	-	4.6	
PO	Lr 1,2c,3,9,16	3.0	3.5	0.8	-	
PM	Lr 1,2c,3,9,26	2.0	1.2	0.8	-	
PR	Lr 1,2c,3,9,16,26	4.1	8.3	3.3	1.5	
PP	Lr 1,2c,3,9,24,26	1.0	1.2	2.5	-	
P		24.4%	13.7%	19.9%	53.7%	
FT	Lr 2c,3,9,16,24,26	4.1	3.5	4.2	4.6	
FO	Lr 2c,3,9,16	2.0	1.2	0.8	-	
F	*************	6.1%	4.7%	5.0%	4.6%	
BB		4.1	4.7	1.6	-	
CT	Lr 3,9,16,24,26	3.0	1.2	-	- T	

* Other race groups recorded frequency less than 1%.

Discussion

Leaf rust of wheat, in particular, has been the main cause of eliminating many cultivars, e.g. cvs. Giza 139, Mexipak 69, Super X, Chenab 70 and Giza 160, because of their susceptibility under field conditions. Moreover, some wheat cultivars were discarded very shortly after their release such as cvs. Giza 139 and Giza 160 (Gomaa, 1978 and Nazim et al., 1990). The failure of such genotypes was mainly due to the dynamic nature in population of the causal organism which produces virulences able to breakdown their resistance.

Generally, new races of leaf rust develop by mutation (Park et al., 2002); heterokaryosis (Kolmer, 1992); recombination (McIntosh, 1992 and Park et al., 1999); migration (Park et al., 1995) and selection of virulence against resistance of the grown wheat varieties in the region (Kolmer, 2005).

The change occurring in virulence of leaf rust population is mostly influenced by new varieties with known or unknown resistance genes and also by a biotic factor, e.g. temperature and rainfall (Nazim and Clifford, 1983 and Manninger, 2001).

^{**} Samples were collected in Egypt during 2003/04-2006/07 and tested during the abovementioned seasons.

It was previously reported by Melchers (1932) and Abdel-Hak et al. (1966) that the alternate host (*Thalictrum* spp.) could not be found in Egypt and that the urediniospores cannot survive in the summer in Egypt due to the high temperature (Nazim et al., 2003 and Abd EL-Malek, 2003) indicating that the initial inoculum comes from external sources (Abdel-Hak et al., 1966; Saari, 1976 and Nazim et al., 2003).

McVey et al. (2004) indicated that wheat leaf rust population in Egypt is made of a great diversity. Moreover, field observations in Egypt showed that the absence of alternate host, volunteer plants and stubs confirm that the source of initial inoculum usually comes from the neighbouring countries, carried by the prevailing winds. Therefore, the present work was concerned with the diversity in population of the causal organism and the frequency of the different virulences. Also this work included greenhouse studies to estimate the frequency of virulences of the causal organism against a set of 20 monogenic lines and some wheat cultivars in order to study the shifts in virulence in the different seasons. Evaluation of the effectiveness of *Lr* genes was included to serve the national breeding programs for leaf rust resistance.

From the results obtained during the four seasons (2004/05 - 2007/08), it was found that the occurrence of virulence frequencies against Lr 9, Lr 21 and Lr 24 were very low but against Lr 3 ka, Lr 36 and Lr 10 were relatively moderate. Shifts in virulence frequency were noticed with Lr 2a, Lr 16, Lr 11, Lr 17, Lr 18 and Lr 26 against the tested isolates. No shifts occurred with Lr 's 1, 2c, 3, 3ka, 9, 24, 26, 30, 10, 21. 14b, 15, 36 and 42. Thus, Lr 9, Lr 21 and Lr 24 were the most effective genes, followed by Lr 3 ka, Lr 10 and Lr 36. Similar results were reported by Nazim et al. (1976); Nazim et al. (1983); Sherif et al. (1996) and Imbaby and Ageez (1998).

Concerning the occurrence of virulence against the tested wheat varieties, results showed different virulence frequencies during the period of study due to the different prevailing races and environmental conditions, as well as the genetic makeup of the different genotypes. However, cv. Gemmeiza 9 showed moderate virulence frequency during the four seasons, whereas, cvs. Gemmeiza 10 and Sakha 94 gave moderate occurrence of virulence in the first three seasons, but lower in the fourth one, reflecting a shift in their reactions.

Generally, the use of wheat genotypes with genetic resistance to leaf rust is the most practical method of controlling the disease. Meanwhile, the use of genotypes with specific genes for resistance selects pathotypes that have virulence to the resistance genes (Manninger, 2001; Long et al., 2002 and Kolmer and Ordoñez, 2007).

McVey et al. (2004) suggested that the use of resistance genes in Egypt has had a strong selective effect on virulence in the pathogen population during the same season. Also, Kolmer et al. (2005) stated that the use of wheat cultivars with leaf rust seedling resistance genes has selected leaf rust phenotypes with resistance to genes Lr 9, Lr 16, Lr 17, Lr 24 and Lr 26 in the United States in 2003.

The physiologic races of *P. triticina* that were identified as major race groups showed different virulence formula during the four seasons (2005–2008). Race group T - - - - was the most dominant race; followed by race group P - - - - , race group B - - - - and race group C - - - - . Race groups TR - - - and TP - - - occurred in lower percentages in the first three seasons and disappeared in the fourth season. On the other hand, race groups PL- - -, PQ- - -, PM- - - and PP- - in addition to race group FQ - - - and race group BB - - - disappeared in the fourth season. Race group CT - - - disappeared in the last two seasons of the study (2006/07–2007/08). Moreover, race group TT - - - showed low percentage of isolation and race group PT - - - showed higher percentage of isolation, reflecting a shift in their occurrence in the fourth season (2007/08).

The difference in virulence appearance and / or disappearance depends on the source of inocula carried to Egypt by northern winds (Nazim et al., 1976).

Concerning virulence formula of the identified race groups, it was noticed that $Lr\ 2c$, $Lr\ 3$ and $Lr\ 9$ were inactive against all identified races during the period of study, whereas, $Lr\ 1$, $Lr\ 16$, $Lr\ 24$ and $Lr\ 26$ were active against certain races and inactive against others.

Shifts in physiologic specialization between leaf rust and resistance genes probably reflect the use of certain Lr genes in wheat cultivars (Watkins, 2001). Thus, wheat varieties should be supplied by effective genes for leaf rust resistance to avoid any expected epidemics.

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تكرار ظهور السلالات الممرضة ومعادلة القدرة المرضية لمرض صدأ أوراق القمح وتعريف السلالات القسيولوجية للفطر المسبب في مصر خلال الفترة ٤٠٠٢/٥٠٠٢ – ٧٠٠٢/٨٠٠٧ محمد نظيم* ، مديح محمد علي** ، إخلاص شفيق*** ، نبوه ابراهيم عبد الملك***

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يعتبر مرض صدا أوراق القمح من الأمراض الرئيسية في مصر والذي يظهر سنويا بدرجات مختلفة من التكرار للسلالات الممرضة – وفي هذا البحث جمعت عينات أوراق القمح المصابة بالمرض من جهات متفرقة من الوجة البحرى خلال أربعه مواسم ٢٠٠٤/٢٠٠٣ – ٢٠٠٢/٢٠٠٢.

وقد تم عزل المسلالات الممرضة باتباع طريقة البثرة الفردية – وأخذت بيانات الصدا بطريقة طرز الإصابة ضد عشرين سلالة قمح حاملة لجينات فردية المعادمة وكذاك بعض اصناف القمح المحلية – وقد تم تسجيل المعادلة المرضية الما لكل سلالة مع تسجيل غياب وظهور المسلالات الفسيولوجية والتغير في ظهور هذه السلالات ونسب عزلها. وقد سجلت اعلى نسب للإصابة على سلالات القمح الحاملة لجينات المقاومة المفردة أرقام سلالات القمح الحاملة لجينات المقاومة المفردة أرقام بلالات المتارة و الله المسلالات المتارة و والصنف جميزة و اقلها في نسب الإصابة و كانت مجاميع السلالات الفسيولوجية --- TT و--- PT و--- PT اكثر السلالات سيادة و قدرة على إصابة مسلالات القمح الحاملة لجينات المقاومة الفردية بارقام و 1, 2c, 3, 9.