

RESPONSE OF SEVEN EGYPTIAN WHEAT CULTIVARS TO LEAF RUST INFECTION (*PUCCINIA TRITICINA*)

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ABSTRACT: Leaf rust disease, caused by *Puccinia triticina* f.sp. *tritici*, is one of the most important wheat diseases in Egypt and worldwide. Growing resistant cultivars is still the most economical and environmentally safe control method. Seven Egyptian wheat cultivars were evaluated for their levels of adult plant resistance to leaf rust infection, under field conditions. Experiments were carried out at two locations i.e. Sids and Kafer El-Hamam and three successive growing seasons i.e. 2010/11, 2011/12 and 2012/13. Over the three years and two locations of the study, Misr-1, Misr-2 and Giza-168 showed high levels of adult plant resistance to leaf rust. They exhibited low percentages of rust severity, low rates of disease increase (*r*-values) and low values of area under disease progress curve (AUDPC). In contrast, Sids-1 and Gemmeiza-11 wheat cvs. as well as the check variety Morocco exhibited high rust severity percentages, high *r*-value estimates and high AUDPC values. On the other hand, wheat genotypes which characterized by a high level of adult plant resistance to leaf rust, under field conditions, proved to have more than one gene to leaf rust resistance i.e., Misr-1 has four genes; Lr2a, Lr3bg, Lr27 and Lr35; Misr-2 has six genes; Lr2a, Lr3bg, Lr27, Lr35, Lr36 and Lr43 and Giza-168 has five genes; Lr3bg, Lr13, Lr27, Lr35 and Lr36. Accordingly, these wheat genotypes known to carry adequate and high levels of partial resistance to leaf rust at the two locations during the three growing seasons of the study. Therefore, they can be considered as a good source of this type of resistance that should be utilized in a breeding program for leaf rust resistance in Egypt.

Key words: Wheat rusts, Durable resistance, partial resistance, gene postulation

INTRODUCTION

Leaf rust is the most widespread foliar disease in Egypt and worldwide. However, the annual yield loss was essentially, due to a time of appearance, a nature of attack, a regular occurrence and prolonged growing season that is prevalent for its development in the wheat growing areas of the world (Khan *et al.*, 1997). Leaf rust causes a considerable annual yield loss on the susceptible wheat cultivars, particularly when infection occurs at early stage of plant growth under suitable environmental conditions for disease incidence and development (Nazim *et al.* 1983; Kolmer 1996 and Nazim *et al.* 2010).

In general, there are two main breeding strategies to control leaf rust resistance: pyramiding of the major resistance genes conferring complete resistance and/or the accumulation of

some minor resistance genes with additive effect to confer partial resistance. However, the resistance conferred by a single gene is frequently overcome by the appearance of the new virulent races in the pathogen population within a short period of time. To obtain a durability of resistance, quantitative resistance, so-called partial or slow rusting resistance, is preferred, in which the infection is not completely stopped but the spread and development of the disease is delayed during the growing season.

In contrast, the use of single race-specific resistance genes with major phenotypic effects has rarely provided long lasting or durable resistance. However, in a way analogous to partial resistance breeding strategy, the combining or pyramiding of resistance genes into individual cultivar has had success for long lasting resistance, particularly in situations where the pathogen

does not reproduce sexually, as in the case of wheat leaf rust pathogen in Egypt. Few or little informations were available for the expression and genes/conditioning type of resistance (PR) in the local wheat cvs., commonly grown in Egypt. The present study was, therefore, carried out to estimate the level of adult plant resistance. Also the the present work aimed to determine the partial resistance to leaf rust in seven local wheat cvs. under different field conditions in Egypt. In addition to postulate leaf rust resistance genes, that may serve as the resistance factors in the expression of this type of resistance under greenhouse conditions.

MATERIALS AND METHODS

a) Field studies :

A total of seven Egyptian wheat genotypes in addition to Morocco variety (check) were evaluated under field conditions at two locations; Sids and Kafer El-Hamam, during three successive growing seasons; 2010/11, 2011/12 and 2012/13. These genotypes were sown in a complete randomized block design with 3 replicates. The experimental plot consisted of 3 rows (3 m long, 30 cm apart and 5 g seed rate for each row). Each experiment was surrounded by 1 m alley and 1.5 m belt, served as a spreader of leaf rust urediospores. This spreader area was grown by the susceptible varieties, *i.e.* Morocco and Thatcher. The spreader plants were artificially inoculated by a mixture of races (urediniospores) mixed with talcum powder at ratio of 1:20 (v:v) using baby cyclone during late tillering and late elongation stages (Tervet and Cassel, 1951).

Disease severity (DS %) was recorded four times, every 10 days intervals, during the studied growing seasons. The method adopted by Peterson *et al.* (1948) was used to determine the coverage percentage of leaves with rust pustules. The obtained data served in the determination of final

rust severity % (FRS %) as outlined by Das *et al.* (1993), area under disease progress curve (AUDPC), according to Pandey *et al.* (1989) and rate of leaf rust increase (r-value), according to Van der Plank (1963). All data were subjected to statistical analysis using MSTAT-C program. Means were compared by the Least Significant Difference (L.S.D) at 5 % level of probability.

b) Greenhouse studies :

Seven Egyptian wheat cvs. Sids-1, Sids-13, Misr-1, Misr-2, Gemmeiza-10, Gemmeiza-11 and Giza-168 and ten monogenic lines for leaf rust resistance; *Lr2a*, *Lr3bg*, *Lr13*, *Lr27*, *Lr28*, *Lr32*, *Lr35*, *Lr36*, *Lr43* and *Lr45* were tested at seedling stage using 12 pathotypes of *P. triticinia* obtained from collected samples during 2012/2013 growing season. Infection type data were used to postulate gene(s) for leaf rust resistance (Stakman *et al.*, 1962).

All plant materials were grown in 10 cm plastic pots. Each pot contained four genotypes, one in each corner in clockwise order. Inoculation and incubation procedures were carried out according to the method adopted by Stakman *et al.* (1962). Rust reaction was recorded on the first leaf, 12 days after sowing as infection type (IT), *i.e.* R = (0, 0;, 1 and 2) and S= (3 and 4) which were designated as L (low infection type) and H (high infection type) (Johnsen 1961). Genes were postulated using the methods adopted by Browder and Eversmyer (1980) and Statler (1984).

RESULTS

Field reaction of leaf rust on 8 wheat cultivars was recorded as rust severity (%) starting from rust appearance until dough stage. Three epidemiological parameters; final rust severity (FRS %), area under disease progress curve (AUDPC) and rate of disease increase (r-value) were estimated.

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1. Final rust severity (%):

Data presented in Table (1) showed final rust severity (%) to leaf rust of 7 Egyptian wheat cvs. comparing to the check variety (Morocco), at adult stage during the three seasons at the two locations, *i.e.* Sids and Kafer El-Hamam. According to their response (s), they may be divided into three groups. The first group; resistant cultivars in the three seasons: Misr-1 (0.3), Giza-168 (0.89) and Misr-2 (2.73). The second group was the moderately resistant cultivars *i.e.* Sids-13 (10.73) and Gemmeiza-10 (32.77). Meanwhile the third group includes the highly susceptible cultivars during the three seasons; Morocco (86.67), Sids-1(79.17) and Gemmeiza-11(48.33).

2. Area under disease progress curve (AUDPC):

Area under disease progressive curve (AUDPC) was estimated for the tested wheat genotypes at two locations, *i.e.* Sids and Kafr El-Hamam during the three growing seasons (2010/11, 2011/12 and 2012/13). The wheat genotypes tested may be classified into two main groups according to the obtained date in Table

(2) and Fig. (1). The first group includes the highly susceptible genotypes or the fast rusting ones *i.e.*, Morocco (1126), Sids-1 (1099.16) and Gemmeiza-11 (707.66). While, the second group includes the partial resistant genotypes such as Misr-1 (6.83), Misr-2 (47.33), Giza-168 (18.17), Sids-13 (144) and Gemmeiza-10 (299).

3. Rate of disease increase (r-value)

Rate of leaf rust increase (r-value) was estimated by using the data obtained from the sequent rust scores; rust severity (%) at certain time from rust appearance until the early dough stage in the two locations, *i.e.* Sids and Kafr El-Hamam during the three growing seasons; 2010/11, 2011/12 and 2012/13.

According to the data presented in Table (3) and Fig. (2), the tested wheat genotypes could be arranged in two main groups. The first group included five genotypes which exhibited low rates of disease increase (r-value). These cultivars were; Misr-1 (0.015), Giza-168 (0.020), Misr-2 (0.041), Sids-13 (0.055) and Gemmeiza-10 (0.092).

Table 1. Final leaf rust severity (%) on eight wheat varieties grown at two different locations during three successive seasons 2010-2013.

Variety	Final rust severity (%)						Mean
	2010/11		2011/12		2012/13		
	Sids	Kafer El-Hamam	Sids	Kafer El-Hamam	Sids	Kafer El-Hamam	
Sids-1	65	80	80	70	90	90	79.17
Sids-13	33.33	1.8	0.6	26.7	1.13	0.8	10.73
Misr-1	0	0	0	0	1.07	0.8	0.3
Misr-2	0	2.33	0	3.13	2.6	8.33	2.73
Gemm.-10	15.33	50	30	21.3	30	50	32.77
Gemm.-11	50	30	40	70	20	80	48.33
Giza-168	0	0	0	1.67	1.4	2.2	0.89
Morocco	90	80	80	90	90	90	86.67

L.S.D. of varieties at 5% (V)

= 4.521

L.S.D. of environments (years and locations) at 5% (E)

= 4.319

L.S.D. of interaction (V*E) at 5%

= 6.781

Table 2. Area under disease progress curve (AUDPC) on eight wheat varieties grown in two different locations during three successive seasons 2010-2013.

Variety	Area under disease progress curve values						Average of AUDPC
	2010/2011		2011/2012		2012/2013		
	Sids	Kafer El-Hamam	Sids	Kafer El-Hamam	Sids	Kafer El-Hamam	
Sids-1	540	1550	1240	865	1275	1125	1099.16
Sids-13	310	33	20	453	27	21	144.00
Misr-1	0	0	0	0	20	21	6.83
Misr-2	0	66	0	37	28	153	47.33
Gemm.-10	206	187	282	252	305	562	299.00
Gemm.-11	512	662	1175	442	215	1240	707.66
Giza-168	0	0	0	37	21	51	18.17
Morocco	965	925	1600	1115	975	1175	1126.00

L.S.D. of cultivars at 5% (C) = 4.521
 L.S.D. of environments (years and locations) at 5% (E) = 3.127
 L.S.D. of interaction (C*E) at 5% = 6.709

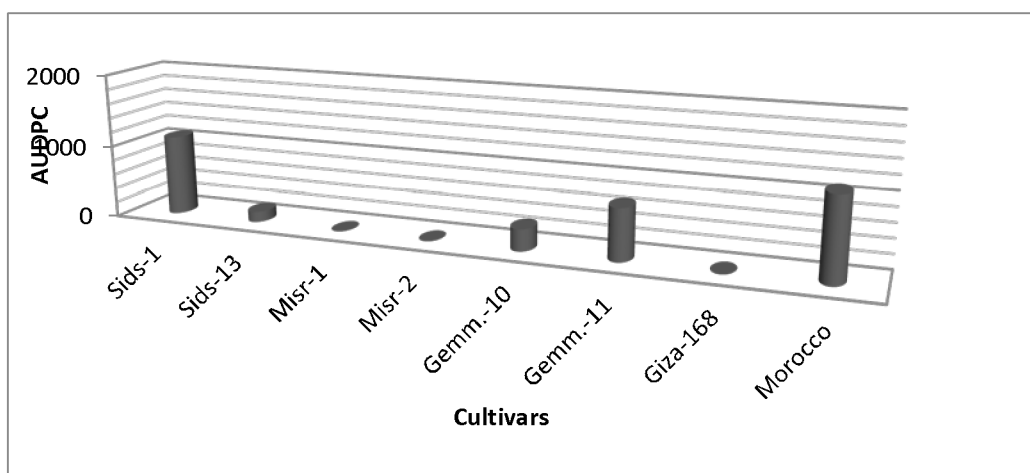


Fig. 1. Mean of area under disease progress curve (AUDPC) for eight wheat varieties grown in two locations during seasons 2010-2013.

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Table 3. Rate of leaf rust increase (r-value) on 8 wheat cvs., grown in two locations during three successive seasons 2010-2013.

Variety	Season / Location / r- value						Average of r-value
	2010/11		2011/12		2012/13		
	Sids	Kafer El-Hamam	Sids	Kafer El-Hamam	Sids	Kafer El-Hamam	
Sids-1	0.119	0.143	0.128	0.144	0.171	0.144	0.142
Sids-13	0.101	0.023	0.142	0.017	0.023	0.023	0.055
Misr-1	0	0	0	0	0.017	0.023	0.015
Misr-2	0	0.089	0	0.041	0.017	0.097	0.041
Gemm.-10	0.092	0.054	0.065	0.085	0.125	0.128	0.092
Gemm.-11	0.124	0.124	0.126	0.065	0.07	0.124	0.106
Giza-168	0	0	0	0.041	0.023	0.057	0.020
Morocco	0.189	0.144	0.162	0.146	0.171	0.171	0.164

L.S.D. of cultivars at 5% (C)

= 0.01482

L.S.D. of environments (years and locations) at 5% (E)

= 0.01284

L.S.D. of interaction (C*E) at 5%

= 0.03631

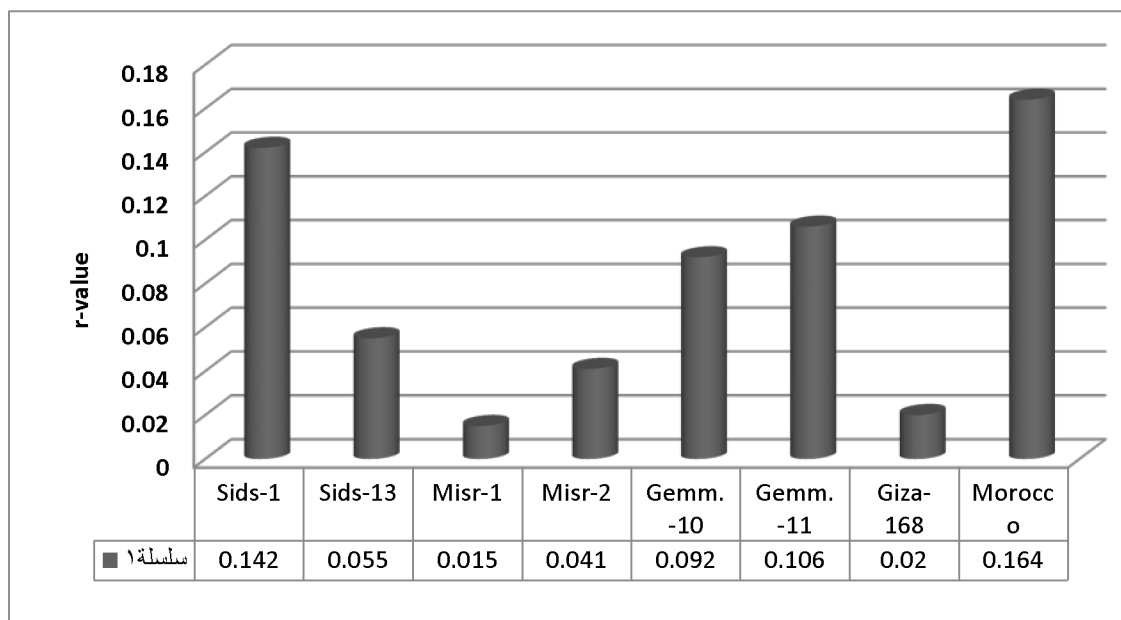


Fig. 2. Mean of rate of disease increase (r-value) on 8 wheat varieties grown in two locations during the three seasons 2010-2013.

On the other hand, the second group included genotypes which exhibited high rate of r-value, *i.e.* Morocco (0.164), Sids-1 (0.142) and Gemmeiza-11 (0.106).

Gene postulation

Matching between infection type (IT) in both local wheat cultivars and Lr genes against the tested physiological races of leaf rust at seedling stage under greenhouse conditions, indicated that postulated genes for leaf rust resistance in genotypes; Misr-1 were *Lr2a, Lr3bg, Lr27* and *Lr35*; in Misr-2 were *Lr2a, Lr3bg, Lr27, Lr35, Lr36* and *Lr43* and in Giza-168 were *Lr3bg, Lr13, Lr27, Lr35* and *Lr36*. Meanwhile, the three genotypes Sids-1, Sids-13 and Gemmeiza-10 probably have a single Lr gene and Gemmeiza-11 has two leaf rust resistance Lr genes (Table 4).

DISCUSSION

Leaf rust (*Puccinia triticina*) was the main cause of eliminating and discarding some wheat genotypes, *i.e.* Giza-139, Mexipak-69, Super-X and Chenab-70, because of their high susceptibility, under field conditions in Egypt (Gomma, 1978). The failure of

such cultivars was mainly due to the dynamic nature of the causal organism, which enables it to produce new virulent races, having the ability to breakdown their resistance. On the other hand, other wheat genotypes, remained resistant for a long period of time from its release and served in agriculture for many years, under their wide cultivations (Khan *et al.*, 2013).

Durable resistance to a disease is a specific term to describe a variety which showed resistance for a long period of time when grown in a large area and under different environments favorable to such disease (Johnson, 2000). This type of resistance has been found in cvs. *i.e.*, Misr-1, Misr-2 and Giza-168. However, a resistance of any variety cannot be described as durable resistance if such variety is grown in a small scale area for a short period of time (Johnson 1984). Therefore, two main tests can be carried out; the first is to test a variety at many locations, while the second is to test it with many races of the pathogen. The two tests give useful information to describe this type of resistance (Boulot 2007).

Table 4. Postulated leaf rust resistance genes (Lr's) that probably present in seven Egyptian wheat cultivars .

Cultivar	Lr genes										Postulated gene	No of genes	Gene frequency (%)
	2a	3bg	13	27	28	32	35	36	43	45			
Sids-1	+	+	+	+	-	+	0	+	+	+	35	1	10
Sids-13	+	+	+	+	-	+	0	+	-	+	35	1	10
Misr-1	0	0	+	0	+	+	0	+	+	+	2a,3bg,27,35	4	40
Misr-2	0	0	+	0	+	+	0	0	0	-	2a,3bg,27,35,36,43	6	60
Gemmeiza-10	-	+	+	+	-	+	0	-	+	-	35	1	10
Gemmeiza-11	+	+	+	0	-	+	0	+	+	+	27,35	2	20
Giza-168	-	0	0	0	-	+	0	0	+	+	3bg,13,27,35,36	5	50

(-) = indicated the absence of such gene in the cultivar; (O) = indicated the presence of such gene in host B and it may have another one and (+) = indicates that either of hosts did not have the same gene.

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Adult plant resistance genes play an important role in the durability of leaf rust resistance in most cultivated wheat, because of the resistance genes expressed at the adult stage, reduce the selection pressure for the pathogen virulence (Kolmer, 1996). Likewise, the genes have proven to be highly effective for about 30 years in Canada and north central United States. Combining two adult plant resistance and seedling resistance genes has provided even greater leaf rust resistance (Kolmer *et al.*, 1991 and Singh 1992). They added that since the extensive use of adult plant resistance genes in that region. Prior to the use of adult plant resistance, leaf rust caused major losses.

To provide an initial description of partial resistance to leaf rust in some local wheat genotypes that are adopted to the Egyptian field conditions, adult-plant resistance (APR) of these genotypes was estimated at two different locations; Sids and Kafer El-Hamam during three successive growing seasons; 2010/11, 2011/12 and 2012/13. Percentage of final rust reaction (FRS %) was recorded for each of the tested genotypes. Accordingly, Misr-1, Misr-2 and Giza-168 were highly resistant. On the other hand, Sids-1 and Gemmeiza-11 as well as the check variety (Morocco) were the highly susceptible. These results are previously supported by the fact that some of the local wheat genotypes, such as Giza-155, Giza-157, Giza-164 and Sakha-61 slightly rusted, showing lower leaf rust severity percentages, under field condition in Egypt (Nazim *et al.*, 1983 and Nazim *et al.*, 1990). In this respect, Das *et al.* (1993) reported that the effective selection for partially resistance genotypes can be practice in the field, based on the final rust severity (FRS %), as a high correlation was found between FRS and the other disease parameters.

Accordingly, adult plant resistance (APR) to leaf rust in the tested wheat genotypes can be accurately measured by using area under disease progressive curve (AUDPC). It considered to be a most convenient and a good reliable estimator for indicating the amount of rust infection, occurred during an epidemic and measured equally well the expression of this type of resistance. Furthermore, AUDPC, in particular, is the result of all factors that influence disease development such as differences in environmental conditions, varieties and population of the pathogen (Nazim *et al.*, 2001; Lal Ahmed *et al.*, 2004 and Singh *et al.*, 2005, Boulot 2007). Furthermore, there are relationship between rust severity, AUDPC and r-value since the cultivars; Misr-1, Misr-2 and Giza-168 which exhibited low rust severity, low rate of disease increase and also low values of area under disease progress curve. On the other hand, the wheat cultivars Morocco, Sids-1 and Gemmeiza-11 exhibited high percentage of final rust severity, high rates of disease increase and high values of area under disease progress curve. Similar results are reported by Omara (2013).

Gene postulation as the most frequent method was carried out to determine the presence of the probable race-specific seedling resistance genes (Lr genes) in a host cultivar at seedling stage. Many researchers have used this method for detecting Lr genes in a group of wheat genotypes (Kolmer, 2003; Oelke and Kolmer, 2004; Wamishe and Milus 2004 and Hysing *et al.*, 2006). Therefore, in this study, seven known genes for leaf rust resistance i.e. *Lr35*, *Lr2a*, *Lr3bg*, *Lr27*, *Lr36*, *Lr43* and *Lr13* in a range of wheat genotypes grown in Egypt.

Pyramiding genes has been suggested as a method to achieve more durable resistance against pathogens with low genetic diversity, high gene/genotype flow and asexual mating

systems (McDonald & Linde, 2002; Hysing *et al.*, 2006). The combination of several effective resistance genes into a single cultivar should extend its period of resistance, *i.e.* Misr-1 has *Lr2a*, *Lr3bg*, *Lr27* and *Lr35*, Misr-2 has *Lr2a*, *Lr3bg*, *Lr27*, *Lr35*, *Lr36* and *Lr43* and Giza-168 has *Lr3bg*, *Lr13*, *Lr27*, *Lr35* and *Lr36*. Therefore, slow rusting or partial resistance has been reported to be more durable resistance than single seedling resistance (Li *et al.*, 2010). The genes *Lr35* and *Lr27* were the most frequent seedling leaf rust resistance genes postulated to be present in Egyptian wheat cvs under study. Therefore, there is relatively inadequate variation in Lr genes carried by wheat cvs. commercially grown in Egypt. Future host selection pressure on the pathogen could be further decreased by rotating genes through time and space by mixtures or regional deployment of cultivars with different effective resistance genes. Nevertheless, classical genetic and molecular marker analyses will be needed to further validate and expand the findings of the present study regarding the Lr genes responsible for both seedling and adult plant resistance to leaf rust in the Egyptian wheat cvs.

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استجابة سبعة أصناف من القمح المصري للعدوى بصدأ الورقة (بكسينيا تريبتيسينا)

جماليات عبد العزيز هرماس

قسم بحوث امراض القمح- معهد بحوث امراض النباتات - مركز البحوث الزراعية

الملخص العربي

يعد مرض صدأ أوراق القمح المتسبب عن الفطر بكسينيا تريبتيسينا من اهم امراض القمح في مصر وجميع انحاء العالم. ومازالت زراعة الاصناف المقاومة الطريقة الاقتصادية والامنة بيئيا لمقاومة ذلك المرض. ولهذا فقد اجري هذا البحث لتقييم سبعة اصناف من القمح المصري في مرحلة النباتات البالغة للوقوف علي مستوي مقاومة كل منهم من لمرض صدأ الاوراق تحت ظروف الحقل خلال ثلاثة مواسم زراعية (٢٠١١ - ٢٠١٣) في محطتي بحوث سدس وكفرالحمام . وبناء علي ذلك فقد اظهرت الثلاثة اصناف: مصر ١ ، مصر ٢ ، جيزة ١٦٨ اعلي مستوي من المقاومة الجزيئية لهذا المرض حيث اظهرت اقل شدة اصابة بالمرض ، اقل معدل تزايد المرض (value) و اقل قيم للمساحة الواقعة تحت منحنى الاصابة المرضي (AUDPC). وعلي العكس فقد اظهرت اصناف القمح سدس ١ ، جيزة ١١ اعلي شدة اصابة مرضية و اعلي قيم لكل من معدل تزايد المرض و المساحة الواقعة تحتي منحنى المرض مقارنة بالصنف موركو (المستخدم للمقارنة). ومن ناحية اخري فقد تميزت الاصناف ذات المستوي العالي من المقاومة لمرض صدأ الاوراق باحتوائها علي اكثر من جين من جينات المقاومة (٤ - ٦ جينات) حيث احتوي الصنف مصر ١ علي اربع جينات للمقاومة وهم *Lr2a, Lr3bg, Lr27, Lr35* ثم الصنف مصر ١ علي ست جينات للمقاومة وهم *Lr2a, Lr3bg, Lr27, Lr35, Lr36, Lr43* ثم الصف جيزة ١٦٨ علي خمس جينات للمقاومة وهم *Lr27, Lr35, Lr36, Lr3bg, Lr13*. وبناء علي ذلك فإن هذه الاصناف تحمل مستويات عالية من المقاومة الجزيئية لمرض صدأ الاوراق في طور النباتات البالغة تحت ظروف الحقل بمحطتي البحوث الزراعية سدس وكفرالحمام وخلال مواسم الزراعة تحت الدراسة ويمكن الاستفادة بها كمصادر جيدة لذلك النوع من المقاومة من خلال برنامج التربية لمقاومة ذلك المرض في مصر.