J. of Plant Protection and Pathology, Vol. 1 (2): 75 - 85, 2010

IDENTIFICATION OF PHYSIOLOGIC RACES OF *Puccinia triticina* AND POSTULATED GENES OF RESISTANCE IN CERTAIN EGYPTIAN COMMERCIAL WHEAT CULTIVARS

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ABSTRACT

The annual survey of 2006/2007 wheat growing season revealed the presence of thrity two physiologic races of wheat leaf rust fungus (*Puccinia triticina* Eriks.). The more frequent race was PTTS (23.07%) followed by race PTTT (12.30%), race TTTS (9.23%), race TTTT (6.15%), race PTTP (4.61%) and the two races PSTN and PTKS which were represented by (3.07%) each. The rest of races were represented by (1.53%) each. However, the more effective leaf rust resistance genes were i.e. Lr2a (73.84%), Lr2b (61.53%) and Lr36 (50.76%).

On the other hand , the highest virulence were observed with Lr's i.e., 10; 11, 14b, 17; 16, 21, 30; and 2c, 3, 24 which were represented by 100% susceptibility; 98.46%; 96.92% and 95.38%, respectively. Leaf rust resistance gene(s) probably present in four Egyptian commercial wheat cultivars in comparison with nineteen leaf rust near-isogenic lines against 65 isolates, the cultivars Gemmiza 10, Giza 168 and Sakha 94 probably have Lr's i.e. 9, 16, 24, 11, 17, 30 and 14b.

On the other hand, the tested Egyptian wheat cultivars were postulated to the lack of Lr2a, Lr2b and Lr36 which were proved to be the more effective genes in the present study. These results will remain an integral part of resistance breeding program and studies relevant to the epidemology and evaluation of virulence to leaf rust pathogen populations.

Keywords : Annual survey **,** avirulence **/** virulence formulae , gene efficacy , leaf rust pathotypes(Pt) , Physiological races , postulated genes , wheat leaf rust near – isogenic lines (NIL**'**s)

INTRODUCTION

In Egypt, wheat rusts are the most common and dangerous diseases on wheat crop. Leaf rust in particular was the cause of eradicating and discarding many wheat cultivars in Egypt, because of their susceptibility under field conditions. However, it has a wide spread on most of the commercial wheat cultivars under Egyptian conditions. Generally, infection might be increased in the late sowing causing high losses in grain yield which reached about 23% on some cultivars (Nazim *et al.*, 1983). To increase leaf rust resistance, breeders attempt to incorporate more than one of the leaf rust near-isogenic lines in the commercial wheat cultivars to face the dynamic nature of causal organisms (Roelfs, 1988). These genes gave us the major importance to facilitate the development and improvement of resistant cultivars to manage leaf rust (Mc Vey and Long, 1993). No doubt that, Flor (1955) was the first to apply the gene postulation technique. However , it was developed to be applied in rust diseases of small grains (Statler, 1984; Modawi *et al.,* 1985 and McVey, 1992). Gene postulation utilized the principles of gene-for-gene, specificity to assign the most resistant lines (Kolmer, 1996).

The main objective of the present investigation are 1) to survey and identify physiologic races of wheat leaf rust in Egypt during 2007/2008 growing season, 2) to postulate leaf rust resistance genes at seedling stage under greenhouse conditions in four Egyptian commercial wheat cultivars together, with twenty near-isogenic lines (NIL's) against sixty five isolates of *Puccinia triticina*.

MATERIALS AND METHODS

Infected leaf rust materials were collected during 2006/2007 growing season from both wheat commercial fields and rust trap (trap nurseries) growing in most Governorates of Arab Republic of Egypt i.e. El-Behera, Garbia, Dakahlia , Damietta, Sharkia, Kalubia , Kafr El-Shiekh, El-Menia, Assuit and Sohag. The scientific application were carried out at both wheat rust laboratories and governed leaf rust greenhouse located at Giza-Orman. Egypt.

The collected samples were kept at room temperature (18 -24°C) overnight to be dried off then the samples were kept in glassine envelopes (8 × 15 cm) and stored in refrigerator at 2 -5°C. These samples were purified and multiplied on the susceptible check cultivars i.e. Thatcher, morocco, Triticum, Spelta Saharensis and Giza 139.

Wheat leaf rust near-isogenic lines (NIL's) listed in Table (1) were used throughout this study. Such seed were kindly supported by wheat germplasm unit located at centro internationale de Mojeramento de maize y Trigo (CIMMYT) at Mexico. While, Egyptian commercial wheat cultivars were kindly supported by wheat Breeding Research Section , Giza (ARC).

Table (1): The tested wheat leaf rust near-isogenic lines (NIL's), genome location, their origin and linkage to other wheat rust diseases applied in the present work.

	Lr gene	Genome location	Origin of seed resources	Linkage
1	1	-	Malakof	
2	2a	5D	Webster	
3	2b	2DS	Carina	
4	2c	2DS	Brivit	
5	3a	6BL	Democrat	
6	3ka	6BL	Klien Aniversario	
7	9	6BL	Triticum Umbellulatum	
8	10	1AS	Lee	
9	11	2A	Hussar	
10	14b	7BL	Bowie	
11	15	2DS	Kenya 1-12 E	
12	16	2BS	Exchange	To Sr23
13	17	2AS	Klien Lucko	
14	18	5BL	T. timophevie	
15	21	1DL	T. tauchii	To Sr24
16	24	3DL	A. elongatum	To Sr31, Yr9
17	26	IBL	Imperial rye	
18	30	4BL	Terenzio	
19	36	6BS	T. speltoides	
20	42	1D	bag	

Lr21 = Lr40

Either of the tested cultivars or (NIL's) were sown into six cm diameter plastic pots filled with peetmoss, perLit and vermiculate, in a specific greenhouse to avoid any contamination. Irrigation, fertilization etc. were performed according to the technical recommendation followed in this regard.

Rust inoculation technique: The rubbing technique was used for inoculation, in which a single pustule was multiplied on the susceptible check cultivars, then rubbed on both of sets of near-isogenic lines (NIL's) and Egyptian commercial wheat cultivars. However, the inoculated seedling were inocubated in dew chamber at (18 - 25°C) in darkness for twenty four hours, and transferred to the permanent benches in the greenhouse for fifteen days in which the day light was available and temperature ranged between 15 - 25°. After twenty one days of sowing, seedlings, were prepared for scoring of infection types according to (Stakman et al., 1962).

The infection types were performed according to the method adopted by Mains and Jackson (1926), in which 0 = (no visible symptoms on the leaf), 0; = (hypersensitive necrosis), 1 = (minute uredinia surrounded by necrotic area), 2 = (medium uredinia surrounded by necrotic or chlorotic area), 3 = (large uredinia surrounded by chlorotic area), 4 = (large uredinia without any chlorosis or necrosis area) and X = (resistant and susceptible reactions are present together on the leaf blade). The data were transmitted to L (R) or H (S), since 0, 0; and 2 are considered resistant or having low reaction, however 3, 4 and X are considered susceptible or having high reaction.

The recent pathotype nomenclature system: to determine the nomenclature of the tested isolates, the four sets of NIL's i.e. 1, 2a, 2c, 3; 9, 16, 24, 26; 3ka, 11, 17, 30 and 10, 18 , 21, 2b were used and applied according to the letters in Table (2) adopted by Long and Kolmer (1989), Statler (1984), Statler et al. (1982). Also, infection types of the rest of (NIL's) i.e. 20 lines in addition to the tested commercial cultivars were recorded for leaf rust reaction.

Resistant gene(s) probable: near isogenic-lines and Egyptian commercial cultivars were both tested at seedling stage against sixty five isolates of *Puccinia triticina*. Rust reaction was demonstrated as low infection type (LIT) or high infection type (HIT). Then a matching was performed between cultivars and (NIL's), also, matching between Egyptian commercial cultivars for common gene(s) all together, were performed according to the method adopted by Statler (1984). These tables were expressed in terms of -, -0, +, 0 as previously mentioned.

Probability of resistance gene(s) incidence: the method mentioned by Statler (1984) was followed, in which a matching was applied for four tested commercial cultivars and twenty near-isogenic lines for leaf rust against sixty five isolates of *Puccinia triticina* to probable resistant genes at seedling stage in commercial wheat cultivars. Also, matching commercial cultivars with them, according to the following categories:

(-) = the cultivar has HIT : LIT in the near-isogenic line. The cultivar may have genes other than those tested.

(-0) = means a typical reaction of both the single gene line and the tested wheat cultivar "the cultivar carrying the same gene".

(+) = both of the tested wheat cultivar and near-isogenic line have not the same gene.
(0) = the cultivar has LIT : HIT in the near-isogenic line. The cultivar may have the same gene and genes other than it.

Table (2): Code (Pt) for the sixteen north American differential hosts for *Puccinia triticina* (Pt) in ordered sets of four [*].

		Infection types produced on near-isogenic lines			
	Host set 1:	1	2a	2c	3
Pt	Host set 2:	9	16	24	26
Code [a]	Host set 3:	3ka	11	17	30
	Host set 4:	10	18	21	2b
B		L	L	L	L
C		L	L	L	H
D		L	L	H	L
F		L	L	H	H
G		L	H	L	L
H		L	H	L	H
J		L	H	H	L
K		L	H	H	H
L		H	L	L	L
M		H	L	L	H
N		H	L	H	L
P		H	L	H	H
Q		H	H	L	L
R		H	H	L	H
S		H	H	H	L
T		H	H	H	H

*** (cf) Long and Kolmer (1989)**
a Pt code consists of the designation for set 1 followed by that for set 2, etc.
L = low infection type H = high infection type

RESULTS

Avirulance/virulence formulae and their frequency:

Data presented in Table (3) indicated the virulence formulas of thirty two tested pathotypes resulted from sixty five isolates and their frequency. These data demonstrated the presence of 32 pathotypes (physiologic races) i.e. PTTS was repeated (23.07%), followed by PTTT was frequent eight (12.30%) , TTTS was replicated six times (9.23%), TTTT was frequent four (6.15%), PTTP was replicated of three (4.61%) , PSTN and PTKS were repeated twice each (3.07%). the rest of the pathotypes were recorded once each (1.53%). Furthermore, the North American nomenclature method revealed that some physiologic races (pathotypes) had numerous numbers of isolates i.e. PTTS, PTTT, TTTS and TTTT contained 15, 8, 6 and 4 isolates, respectively. It could be noticed that isolate no. 32 i.e (TTTT) is considered to be the most virulent one, since it exhibited complete virulence to either of the tested Lr's followed by each of no. 8 (KTTT), 25 (PTTT), 26 (RTTT), 27 (STTT), 30 (TTST), 31(TTTS), which were avirulent to only one Lr. On the other hand, isolates no. 1 (FGSS), 14 (PFFN), were avirulent to 7 Lr's each.

Table (3): Avirulence/virulence formulae of leaf rust pathotypes (Pt) identified in Egypt in 2007/2008.

	No. of pathotypes	Pathotypes	Avirulence/virulence formulae	Virulence frequency (%)
1	(1)	FGSS	1, 2a; 9, 24, 26; 30; 2b/	1.53
2	(1)	FRTT	1, 2a; 24/	1.53
3	(1)	FSTP	1, 2a; 26; 18/	1.53
4	(1)	FTTP	1, 2a; 18/	1.53
5	(1)	FTTQ	1, 2a; 21, 2b/	1.53
6	(1)	FTTS	1, 2a; 2b/	1.53
7	(1)	KTTN	1; 18, 2b/	1.53
8	(1)	KTTT	1/	1.53
9	(1)	MPTS	2a, 2c; 16; 2b/	1.53
10	(1)	MTTP	2a, 2c; 18/	1.53
11	(1)	NQTS	2a, 3; 24, 26, 2b/	1.53
12	(1)	NSTS	2a, 3; 26; 2b/	1.53
13	(1)	NTKS	2a, 3; 3ka; 2b/	1.53
14	(1)	PFFN	2a; 9, 16; 3ka, 11; 18, 2b/	1.53
15	(1)	PJTS	2a; 9, 26; 2b/	1.53
16	(1)	PRTS	2a; 24; 2b/	1.53
17	(2)	PSTN	2a; 26; 18, 2b/	3.07
18	(1)	PSTP	2a; 26; 18/	1.53
19	(1)	PTHS	2a; 3ka, 17; 2b/	1.53
20	(1)	PTKP	2a; 3ka; 18/	1.53
21	(2)	PTKS	2a; 3ka; 2b/	3.07
22	(1)	PTTN	2a; 18, 2b/	1.53
23	(3)	PTTP	2a; 18/	4.61
24	(15)	PTTS	2a; 2b/	23.07
25	(8)	PTTT	2a/	12.30
26	(1)	RTTT	2c/	1.53
27	(1)	STTT	3/	1.53
28	(1)	TSTS	26; 2b/	1.53
29	(1)	TTST	30/	1.53
30	(1)	TTTQ	21, 2b/	1.53
31	(6)	TTTS	2b/	9.23
32	(4)	TTTT	-	6.15
Total	65			

The Lr's on the left side of the slash were written however those on the right were neglected as having susceptible reaction.

Leaf rust resistance gene efficacy %:

Data presented in Table (4) revealed the efficacy of leaf rust resistance gene in controlling pathotypes populations in most governorates of Egypt during 2006/2007 growing season. These data indicated that three genes had the more efficacy in controlling leaf rust pathotypes, which were recorded with Lr's i.e. 2a, 2b and 36. On the other hand, lowest efficacy genes were reported with Lr's i.e. 10, 11, 14b, 17, 16, 21 and 30. However, the rest of the leaf rust near-isogenic lines lied in between the above mentioned, limits.

Data in Table (5) demonstrated that, thirty two pathotypes of *P. triticina* were identified from sixty five isolates by four sets of leaf rust resistance genes. Concerning the matching of four Egyptian commercial wheat cultivars and twenty leaf rust near-isogenic lines against sixty five isolates of leaf rust pathogen (*P. triticina* Eriks). Data in Table (6) indicated the presence of low infection type : high infection type in the (commercial : near-isogenic line)

indicating the inclusion of the Lr within the genetic make up of the commercial wheat cultivar. This was assigned by the symbol (0).

Table (4): Virulence frequency (%) of wheat leaf rust (*Puccinia triticina*) and Lr's genes efficacy (%) within four wheat commercial varieties in Egypt during 2007/2008 growing season.

	Lr's	No. of virulent isolates	Virulence frequency %	Gene efficacy
1	1	57	87.692	12.307
2	2a	17	26.153	73.847
3	2b	25	38.461	61.539
4	2c	62	95.384	4.616
5	3	62	95.384	4.616
6	3ka	59	90.796	9.231
7	9	62	95.384	4.616
8	10	65	100.0	-
9	11	64	98.461	1.539
10	14b	64	98.461	1.539
11	15	54	87.692	12.307
12	16	63	96.923	3.077
13	17	64	98.461	1.538
14	18	52	80.00	20.00
15	21	63	96.923	3.077
16	24	60	92.307	7.692
17	26	56	84.615	15.384
18	30	63	96.923	3.077
19	36	30	46.153	53.846
20	42	52	80.00	20.00

Other group of comparisons showed the presence of low infection type : high infection type and high infection type : low infection types between the commercial and near-isogenic lines against the tested isolates and this would indicate that each of the cultivars may have gene(s) not involved in the other. It was assigned by the symbol (+). Certain groups showed low infection types within the near-isogenic lines, however the commercial wheat cultivars exhibited high infection type in the matching against the isolates. This would indicate the absence of such gene(s) within the commercial cultivars, and would assigned by the symbol (-).

Data in Table (6A) revealed the probable resistance genes may be presented within the genetic back ground of certain commercial wheat cultivars as derived from the last table. These data revealed the existence of 13, 10 and 8 resistance genes in Sakha 94, Gemmiza 10 and Giza 168, in respect. On the other hand , Gemmiza 9 may include nothing of the tested genes (-) or may have genes other than those tested (+).

As regard to the distribution of leaf rust resistance genes within commercial wheat cultivars, data presented in Table (6B) indicated that, Lr9, 11, 14b, 16, 17, 24 and 30 were the most common genes within the Egyptian commercials i.e. Gemmiza 10, Giza 168 and Sakha 94 followed by Lr2c, 15, 18 and 21, exhibited afrequency reached to 50%. Lr1 and Lr26 have afrequency reached to 25%. However, six Lr's out of nineteen were not present in that cultivars, due to the presence of (- or +).

Table (5): Infection types produced by selected pathotypes of *Puccinia triticina* (Pt) isolated from most governorates of Egypt against leaf rust resistance genes and differential sets samples under greenhouse conditions at seedling stage in 2007/2008 growing season.

No.	Pathotypes	Lr genes sets															
		1	2a	2c	3	9	16	24	26	3ka	11	17	30	10	18	21	2b
1	FGSS	L	L			L		L	L				L				L
2	FRTT	L	L					L									
3	FSTP	L	L						L						L		
4	FTTP	L	L												L		
5	FTTQ	L	L													L	L
6	FTTS	L	L														L
7	KTTN	L													L		L
8	KTTT	L															
9	MPTS		L	L			L										L
10	MTTP		L	L											L		
11	NQTS		L		L			L	L								L
12	NSTS		L		L				L								L
13	NTKS		L		L					L							L
14	PFFN		L			L	L			L	L				L		L
15	PJTS		L			L			L								L
16	PRTS		L					L									L
17	PSTN		L						L						L		L
18	PSTP		L						L						L		
19	PTHS		L							L		L					L
20	PTKP		L							L					L		
21	PTKS		L							L							L
22	PTTN		L												L		L
23	PTTP		L												L		
24	PTTS		L														L
25	PTTT		L														
26	RTTT			L													
27	STTT				L												
28	TSTS								L								L
29	TTST												L				
30	TTTQ															L	L
31	TTTS																L
32	TTTT																

Table (6A): Probably present or may be present resistance genes for leaf rust near-isogenic lines within some Egyptian commercial wheat cultivars at seedling stage 2007/2008 growing season.

No.	Cultivars	Probable Lr's* gene(s)
1	Gemmiza 9	-
2	Gemmiza 10	2c, 9, 16, 24, 11, 17, 30, 18, 14b, 15
3	Giza 168	9, 16, 24, 11, 17, 30, 21, 14b
4	Sakha 94	1, 2c, 9, 16, 24, 26, 11, 17, 30, 18, 21, 14b, 15

*** Lr's = leaf rust resistance genes.**

Table (6B): The frequency of identified Lr's genes within Egyptian commercial wheat cultivars at seedling stage in 2007/2008 growing season.

No.	Lr's gene	No. of cultivars carrying Lr's gene	Frequency %
1	1	1	25
2	2a	0	0
3	2b	0	0
4	2c	2	50
5	3	0	0
6	3ka	0	0
7	9	3	75
8	11	3	75
9	14b	3	75
10	15	2	50
11	16	3	75
12	17	3	75
13	18	2	50
14	21	2	50
15	24	3	75
16	26	1	25
17	30	3	75
18	36	0	0
19	42	0	0

Data presented in Table (7) indicated that, matching of commercial wheat cultivars within each other for searching the common gene(s). the cultivars may have genes other than those tested near-isogenic lines and would assigned by the symbol (+).

Table (7): Incidence of culture in the LIT : HIT (low infection type : high infection type) comparisons between cultivars inoculated with sixty five isolates of *Puccinia triticina* f.sp. *tritici*.

Cultivars	Gemmiza 9	Gemmiza 10	Giza 168	Sakha 94
Gemmiza 9		+	+	+
Gemmiza 10	+		+	+
Giza 168	+	+		+
Sakha 94	+	+	+	

+ = indicated either of hosts did not have the same gene

DISCUSSION

Leaf rust caused by Puccinia triticina Eriks (Puccinia recondite Rob. Ex Desm. F.sp. tritici Eriks and Henn) was the first factor in failure of such cultivars, which was mainly due to the dynamic nature in population of the causal organism which produces new virulence having the ability to breakdown their resistance. The virulence combination were designated by a four letter code (Long and Kolmer, 1989). The obtained results demonstrated that sixty five leaf rust isolates gaves thirty two virulence combinations (physiologic races). Race PTTS was the more prevalent one and comprised (23.07%) of the races, followed by PTTT, TTTS, TTTT, PTTP, PTKS and

PSTN were frequently repeated by (12.30%), (9.23%), (6.15%), (4.61%), (3.07%) and (3.07%) in respect. The rest of physiologic races (virulence formulae) were frequently repeated by (1.53%) each. Similar results were recorded by Najeeb et al. (2005) and Youssef (2006).

As for gene efficacy (%) of the tested Lr's, the obtained results indicated the presence of high efficacy which were reported with i.e. Lr's 2a, 2b and 36 which represented by 73.84%, 61.53% and 50.76%, respectively.

Breeding for disease resistance is considered to be the most economic procedure for controlling leaf rust disease. It requires the identification of the prevalent physiologic races of *P. triticina* and component of effective genes conferring resistance which they included to be utilized in better choice within the selected parents to enhance protection.

The obtained results demonstrated the possibility of the identification of thriteen out of twenty of leaf rust near-isogenic lines in four tested commercial wheat cultivars against sixty five leaf rust isolates under greenhouse conditions at seedling stage.

The postulated genes of leaf rust near-isogenic lines within the Egyptian wheat cultivars i.e. Lr's 9, 16, 24, 11, 17, 30 and 14b, probably found in Gemmiza 10, Giza 168 and Sakha94. Lr2c, Lr18 and Lr15 probably presented in Gemmiza 10 and Sakha 94 Lr21 was postulated to be existend in Giza 168 and Sakha 94. Also, Sakha 94 probably included Lr1 and Lr26. However , the rest of the leaf rust near-isogenic lines i.e. Lr's 2a, 3ka, 2b, 36 and 42 probable couldn't be detected in the tested commercial cultivars in this work. Similar results were recorded by (Mc Vey, 1989; Najeeb et al., 2005; Youssef, 2006 and Roelfs and Martens, 1988).

These results are not considered and indication to lack of the other tested cultivars from resistant genes, but they may have genes other than those tested (category +).

The second probability is the presence of such gene in tested cultivars but the existence of suppressors or modifiers prevented their expression (Knott, 1989). This assumption may be the reason behind the genetic behaviour of certain local cvs. toward the tested races.

These results are limited by the number of isolates and available tester lines having single known Lr gene (Statler *et al.,* 1982). However , this would be an effective tool in the disease breeding program for leaf rust resistance.

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تعريف السلالات الفسيولوجية لفطر بكسينيا تريتيسينا والجينات المتوقعة في بعض أصناف القمح التجارية المصرية

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بين الحصر السنوي لموسم ٢٠٠٧/٢٠٠٦ وجود سلالة فسيولوجية من فطر صدأ الأوراق (بكسينيا تريتيسينا) حيث أن السلالة PTTS كانت أكثر تكراراً (٢٣.٠٧%) يليها السلالة PTTT (١٢.٣٠%) ، والسلالة TTTS (٩.٢٣%) ، السلالة TTTT (٦.١٥%) ، والسلالة PTTP (٤.٦١%) ، والسلالتين PSTN ، PTKS كلاً منهما يمثل (٣.٠٧%) ، باقي السلالات كل منها كان يمثل (١.٥٣%) . السلالات الأحادية الجين الأكثر كفاءة في مقاومة صدأ الأوراق كانت Lr2a (٧٣.٨٤%) ، Lr36 (٥٠.٧٦%) ، Lr2b (٦١.٥٣%) . على الجانب الآخر أعلى عدوانية لوحظت مع الجين الأحادى.

الجين مثل Lr10 (١٠٠%) ، والجين Lr11, 14b, 17 كل منها يمثل (٩٨.٤٦%) والجينات Lr16, 21, 30 كل منها يمثل (٩٦.٩٢%) والجينات Lr 2c, 3, 24 كل منها يمثل (٩٥.٣٨%) فى درجة العدوانية.

احتمال وجود جينات مقاومة لفطر صدأ الأوراق في أربعة أصناف قمح تجارية مصرية وذلك بمقارنتها مع تسعة عشرة سلالة أحادية الجين لمقاومة صدأ الأوراق ضد ٦٥ عزلة من المسبب المرضي ، الصنف جميزة ١٠ ، جيزة ١٦٨ ، سخا ٩٤ احتمال أنها تملك الجينات ٩ ، ١٦ ، ٢٤ ، ١١ ، ١٧ ، ٣٠ ، ١٤ ب . أحتمال أن أصناف القمح المصرية التي تم اختبارها في هذه الدراسة ينقصها الجينات التي ثبت أنها أكثر كفاءة في هذا العمل . هذه النتائج سوف تبقى جزء مكمل من برنامج التربية للمقاومة والدراسة المتعلقة بالوبائية وتطور العدوانية في عشيرة المسبب المرضي لصدأ الأوراق .

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