

BIOCHEMICAL AND PATHOLOGICAL CHARACTERIZATION OF LEAF RUST RESISTANCE IN SOME EGYPTIAN WHEAT CULTIVARS

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ABSTRACT: Leaf rust of wheat is caused by the fungus *Puccinia triticina* Eriks. (syn. *P. recondita* Rob. Ex Desm. f. sp. *tritici* Eriks. And Henn.). Six Egyptian wheat cultivars (*Triticum aestivum* L.) (i.e. Giza139, Giza168, Gemmiza7, Gemmiza9, Misr1 and Sakha93) have been used to study pathological and biochemical characterization of leaf rust resistance. The tested six wheat cultivars were screened for seedling reaction and adult plant response against leaf rust pure race (PTSS), under greenhouse conditions. This screening was carried out concerning the infection type, final rust severity and AUDPC. Results indicated that Misr-1 was completely resistant, at both stages. Giza-168 and Gemmiza-9 wheat cultivars recorded low values of the estimated parameters in adult plant reaction. Meanwhile, Giza-139 and Gemmiza-7 were susceptible where they recorded high values of the estimated parameters. Some protein bands are correlated with rust resistance in wheat at molecular weight at about 17 kDa and 12 kDa (for total protein pattern) and at 140 kDa and 34 kDa (for the water soluble protein pattern) mainly for "Misr1" wheat cultivar (completely rust resistance cultivar according to the pathological experiment). Thus it can be said that biochemical and pathological experiments revealed that Misr-1 wheat cultivar is rust resistant. It can be also said that biochemical markers could be used to screen whether a wheat cultivar is rust resistant or not because some protein bands have been identified to be correlated with rust resistance. Thus biochemical markers can be used to save our effort and time to determine the rust resistant and susceptible wheat cultivars.

Key words: Wheat, Leaf Rust, *Puccinia triticina*, Protein markers.

INTRODUCTION

Breed wheat (*Triticum aestivum* L.) is cultivated worldwide as stable food with a global production. Egypt produced 8.5 million tons of wheat grains from 3.214 million feddan in 2013 growing season, while the total consumption was about 18 million tons (USDA 2014). So Egypt imports annually about 10 million tons of wheat grains. The gap between wheat production and consumption is escalating due to ever increasing population. Wheat production is also decreasing due to the attack of certain diseases like rusts, smuts, and powdery mildew ...etc.

Rust diseases are the most important diseases of wheat because of their ability to

move for long distance, and their ability to form new races that can attack resistance varieties. Leaf rust caused by the fungus *Puccinia triticina* Eriks, previously known as *P. recondita* f. sp. *tritici*, is a common disease of bread wheat (Kolmer, 1996). Classification of Egyptian wheat varieties according to their rust resistance has been carried out by some researchers. Abd El-Malik (2011) showed that the cultivars Sids-1, Giza-139 and Giza-160 exhibited the highest rust severity during 2006/07 and 2007/08 growing seasons. Also, Omara (2013) evaluated fifteen wheat cultivars against leaf rust under field conditions and indicated that the wheat cultivars Giza-168, Misr-1, Misr-2, Sakha-94 and Sakha-95

were highly resistant. On the other hand, the wheat cultivars Gemmiza-7, Sids-1, Sakha-61, Sakha-93 and Sakha-69 were the highly susceptible.

The effective resistance genes and virulence patterns of wheat leaf rust under field conditions has been recently studied in different countries such as Iran (Safavi and Afshari 2013), Turkey (Kolmer *et al* 2013) and Egypt as well (Omara, 2013). The leaf rust resistance genes *Lr17*, *Lr18*, *Lr19*, *Lr22a*, *Lr23+*, *Lr25*, *Lr28*, *Lr35* and *Lr36* and the combination of genes *Lr13*, *Lr27*, *Lr31* and *Lr34* together/with other resistance genes were found in the Iranian wheat genotypes while the genes *Lr1*, *Lr3a*, *Lr10*, *Lr14a*, *Lr17a*, *Lr20*, *Lr23*, and *Lr26* were postulated to be present in the Turkish wheat cultivars. In Egypt, Omara (2013) mentioned that the adult plant resistance genes *Lr19* and *Lr25* were the highly resistant to leaf rust followed by *Lr9*, *Lr28*, *Lr29*, *Lr45*, *Lr36*, *Lr18*, *Lr27*, *Lr47*, *Lr46*, *Lr22a*, *Lr34*, *Lr43*, *Lr21* and *Lr24*.

Biochemical markers have been used to study the resistance pattern of wheat genotypes against rust disease. Póš *et al* (2010) used 'Thatcher' wheat cultivar and two of its near-isogenic lines to investigate changes in the protein- and gene expression associate with wheat leaf rust infection and susceptible seedlings using Isoelectric focusing. They detected in association with leaf rust infection altered expression of at least 24 proteins in the near-isogenic lines of the 'Thatcher' cultivar showing differences in the resistance to the fungus. Dilara *et al* (2013) compared six hundred and thirty-seven protein peaks one by one between protein patterns obtained from pathogen-

and mock-inoculated wheat leaf tissue. They reported that 33 proteins were identified in *Pst*-infected plants as compared with mock-inoculated control. Sodium dodecyl sulphate – gel electrophoresis (SDS-PAGE) also has been used by Shaista *et al* (2011) to screen out the molecular weight of gluten subunit and genetic diversity of wheat varieties from Pakistan. They noticed variation in the number and position of bands from one variety to the other, while some bands are common.

This work aimed to study the response of wheat cultivars against leaf rust (brown rust) infection. The response was studied at both pathological and biochemical levels.

MATERIALS AND METHODS

Plant material:

Six Egyptian wheat cultivars (*Triticum aestivum* L.) i.e. "Giza-139", "Giza-168", "Misr-1", "Gemmiza-7", "Gemmiza-9", and "Sakha-93" were used (Table 1). Grains of these cultivars were kindly obtained from Crops Research Institute, Agricultural Research Center, Giza, Egypt. The pedigree, types and origins of the wheat cultivars are presented in Table (1).

Pathological experiments:

The pathological experiments were carried out under greenhouse conditions on both seedling and adult plants using urediospores of (PTSS) pathotype of *Puccinia triticina* f.sp. *tritici*. Urediospores were kindly provided by Wheat Disease Department, Plant Pathology Research Institute, Agricultural Research Center, Egypt during 2012/13 growing season.

Table (1): List of six Egyptian wheat cultivars and their pedigrees.

No.	Cultivar	Pedigree
1	Giza-139	Hindi 90X Kenya B.256.
2	Giza-168	MIL/BUC//Seri CM93046-8M-0Y-OM-2Y-0B
3	Misr-1	OASIS/SKAUZ//4*BCN1312*PASTOR.
4	Gemmeiza-7	CMH74A.630/5X//Seri 82/3 AgentCGM.4611-2GM.-3GM.-1GM.-0cm
5	Gemmeiza-9	ALD"S"/HUAC"S"/CMH74A.630/SX.GM4583-5GM-1GM-0GM.
6	Sakha-93	Sakha 92/TR810328 S 8871-1S-2S-1S-0S

Seedling stage pathological experiment:

Five seedlings of each cultivar grown in 7cm diameter plastic pots were inoculated in the first leaf stage with urediospores of (PTSS) pathotype of *Puccinia triticina* f.sp. *tritici*. Urediospores were mixed with talcum powder of 1:20 (v/v) according to (Tervet and Cassel, 1951). Three pots (replicates) were used for each cultivar. After 24 hr. of incubation in dew chamber less than 100% relative humidity, the inoculated seedlings were shifted to glass house benches where the temperature at $22 \pm 2^{\circ}\text{C}$ with approximately 80% relative humidity. Wheat seedlings were checked daily for pustules appearance. Infection types were recorded according to Stakman *et al.* (1962), 0, 0_i, 1 and 2 (Resistance infection type), 3 and 4 (Susceptible infection type) as shown in Table (2).

Adult stage pathological experiment:

Twenty five wheat grains from each cultivar were grown in 25 cm diameter pots. After germination, the plants were thinned to 10 plants/pot. Three pots (replicates) were used for each cultivar. Artificial inoculation was carried out at booting stage as

mentioned by Large (1954). Plants were dusted with urediospores of (PTSS) pathotype of *Puccinia triticina* f.sp. *tritici*. Leaf rust severity (%) was estimated in all tested cultivars according to the modified Cobb's scale (Peterson *et al.*, 1948). Disease severity was recorded at 10 days intervals, after rust symptoms firstly appeared on each of the tested cultivars and until the plants were dried.

Area Under Disease Progress Curve (AUDPC) value was calculated for each cultivar using the equation of Pandey *et al.* (1989):

$$\text{AUDPC} = D \left[\frac{1}{2} (Y_1 + Y_K) + (Y_2 + Y_3 + \dots Y_{K-1}) \right]$$

Where: D = days between two consecutive recordings (time intervals),

$Y_1 + Y_K$ = sum of the first and the last disease scores and

$Y_2 + Y_3 + \dots + Y_{K-1}$ = sum of all in between scores.

Data were analyzed in combined analysis of Randomized Complete Block Design (R.C.B.D.) to study the interactions between tested cultivars (Snedecor and Cochran, 1967).

Table (2): The infection types of wheat leaf rust reactions adopted by Stakman *et al.* (1962) at seedling stage.

Infection type	Symptoms
0 Low R*	No uredia or other macroscopic sing of infection
0 _i	No uredia, but hypersensitive necrotic or chlorotic flecks of varying size present
1	Small uredia often surrounded by necrosis
2	Small to medium uredia often surrounded by chlorosis or necrosis. T may be associated with chlorosis
3 High S**	Medium- sized uredia that may be associated with chlorosis or rarely necrosis
4	Large uredia without chlorosis or necrosis

Biochemical analysis:

Sodium Dodecyle Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique was used to study the protein banding patterns of six varieties of wheat (*Triticum aestivum* L.). Both total protein and water-soluble protein were extracted from leaves according to the method of Laemmli (1970). The protein was isolated from infected and non-infected leaves with the leaf rust spores of the six tested wheat cultivars; so that a total of 12 samples were used for this analysis. The extracted protein was separated on SDS-PAGE and stained using Commassie Brilliant blue pigment.

The gels were photographed with digital camera and the photos were handled with Adobe Photoshop 9 (CS2) software in order to adjust the contrast and the brightness then gels were scored as 0/1 for the absence/presence of bands, respectively. Specific bands have been determined for specific cultivars and correlation between the pathological experiments and the biochemical markers has been made according to the specific protein bands. Cluster analysis using NTSYSPC v2.1 software (Rohlf, 1998). Similarity coefficient matrices were calculated using simple matching similarity algorithm (Sokal and Sneath, 1963). Phylogenetic dendrogram

was constructed using the UPGMA method (Sneath and Sokal, 1973).

RESULTS AND DISCUSSIONS

The tested six wheat cultivars were screened for seedling reaction and adult plant response against leaf rust pure race (PTSS), under greenhouse conditions. This screening was carried out concerning the infection type, final rust severity and AUDPC. Results given in Table (3) indicated that "Misr-1" was completely resistant at both stages whereas it recorded zero infection type and rust severity values. "Giza-139" and "Gemmiza-7" wheat cultivars recorded susceptible reactions at both stages. Meanwhile, "Giza-168", "Gemmiza-9" and "Sakha-93" recorded varied reactions between seedling and adult stages. At seedling stage, the susceptibility reactions were recorded by "Giza-168" and "Sakha-93" wheat cultivars. While the resistant reaction was recorded by "Gemmiza-9". At adult stage, the moderate susceptibility reactions were recorded by "Gemmiza-9" and "Sakha-93". While the moderate resistant reaction was recorded by "Giza-168". Statistical analysis indicated that there were significant differences between cultivars for all parameters.

Table (3): Leaf rust infection types on seedling stage and final rust severity and area under disease progress curve (AUDPC) on adult stage against (PTSS) race of *Puccinia triticina* f.sp. *tritici*, under greenhouse conditions.

Cultivar	Seedling reaction Infection type	Adult plant reaction	
		Final rust severity	AUDPC
Giza-139	4.00 a	46.66 S	640.50 a
Giza-168	3.00 b	0.400 MR	7.5000 e
Misr-1	0.00 d	0.000	0.0000 e
Gemmiza-7	3.00 b	36.66 S	423.83 b
Gemmiza-9	1.33 c	10.00 MS	123.16 d
Sakha-93	3.33 b	20.00 MS-S	193.16 c
L.S.D. at 5%	0.5927	6.163	69.07

S = Susceptible; MR = Moderately resistant; MS = Moderately susceptible

Data shown in Figure (1) strengthened those given in Table (3) where Giza-139 and "Gemmiza-7" cultivars recorded the higher values of AUDPC (640.5 and 423.83, respectively). These values indicated the fast rusting reaction of these cultivars. "Giza-168" and "Gemmiza-9" cultivars were characterized by slow rusting reaction, where they recorded the lowest values of AUDPC (7.5 and 123.17, respectively). The recorded resistance reaction of "Misr-1" and "Giza-168" may be due to the presence of five resistance *Lr* genes, while the low value of final rust severity which recorded by "Gemmiza-9" may be due to the presence of four resistance *Lr* genes (Abou-Elseoud *et al.*, 2014). Our results are in contiguous with those finding by McVey *et al.* (2004), Najeeb *et al.* (2005) and Imbaby (2007). They observed the same results concerning rust resistance of Egyptian wheat cultivars.

Biochemical analysis:

SDS-PAGE technique was used to study

the protein banding patterns of six wheat cultivars. Total leaf protein as well as the water soluble protein was extracted from leaves of the six cultivars (rust infected and non-infected as a control) using Lamealli modified method. The extracted protein was separated on SDS-PAGE and stained using Commassie Brilliant blue pigment. According to the protein analysis, a total of nine bands were observed in the water-soluble protein pattern for all the studied cultivars while 14 bands were observed from the total protein pattern overall the cultivars (Figures 2 and 3). Some bands were identified that may be correlated with rust resistance in wheat; whereas a band at about kDa molecular weight 140 kDa i has been noticed from the water soluble protein pattern of "Misr1" wheat cultivar (completely rust resistance cultivar according to the pathological experiment). Another band has been identified also at molecular weight of 34 kDa for the same cultivar (Figure 2).

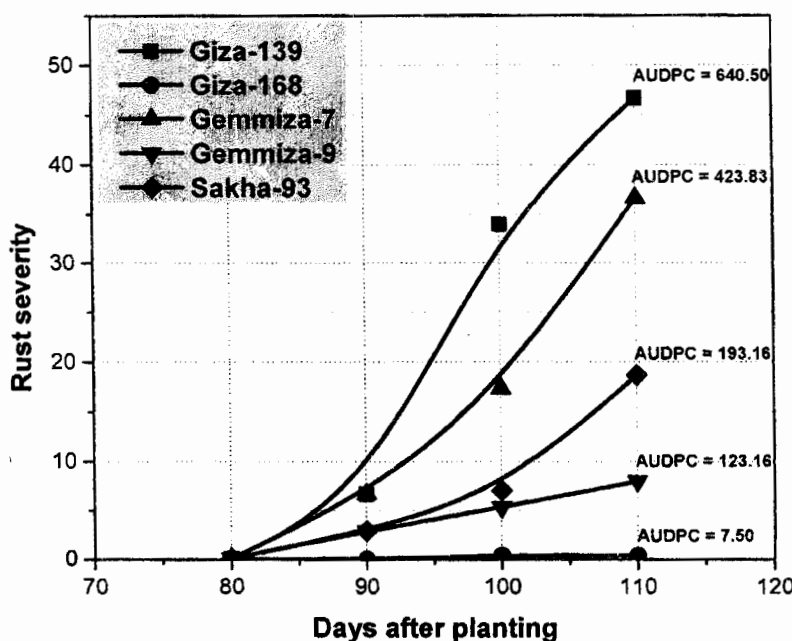


Figure (1): Area Under Disease Progress Curves (AUDPC) for *Puccinia tritici* f. sp. *tritici* on six adult wheat cultivars, during 2012/13 growing season. Misr-1 recorded zero value.

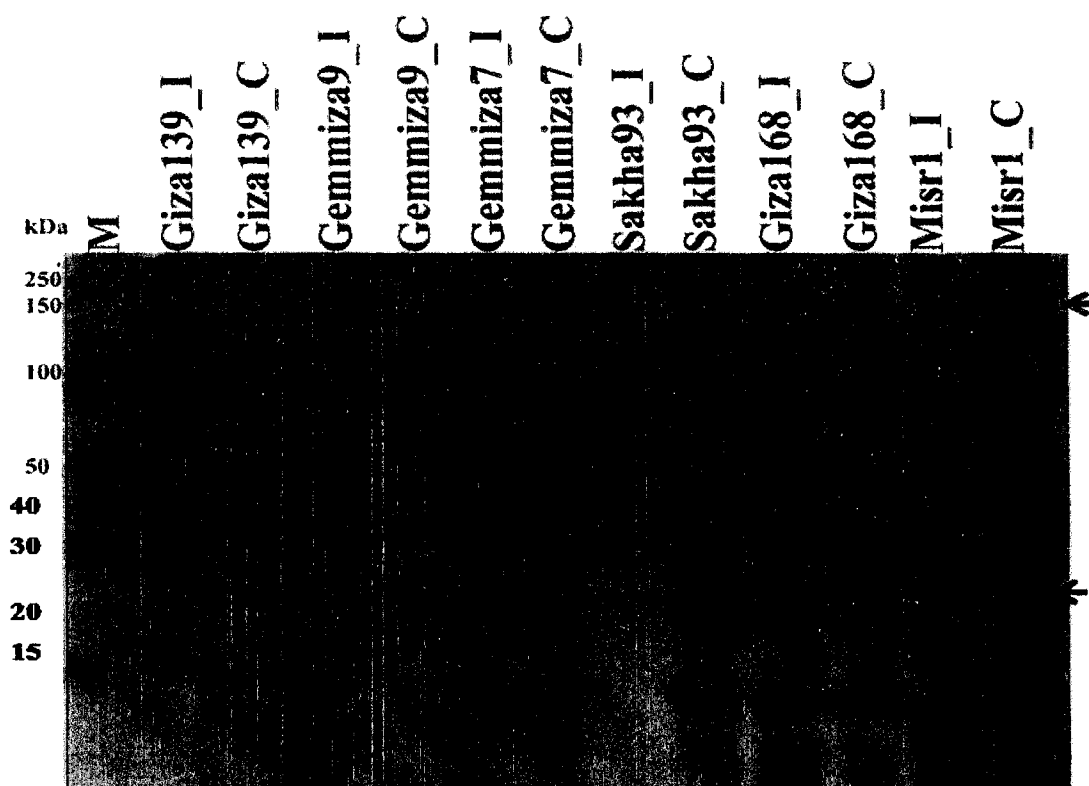


Figure (2): Leaf water soluble protein pattern of twelve leaf rust infected and non-infected wheat (*Triticum aestivum* L.) cultivars separated on 15% SDS-PAGE. The black arrows show positive protein marker. I: refers to infected cultivar while C: refers to the control cultivar.

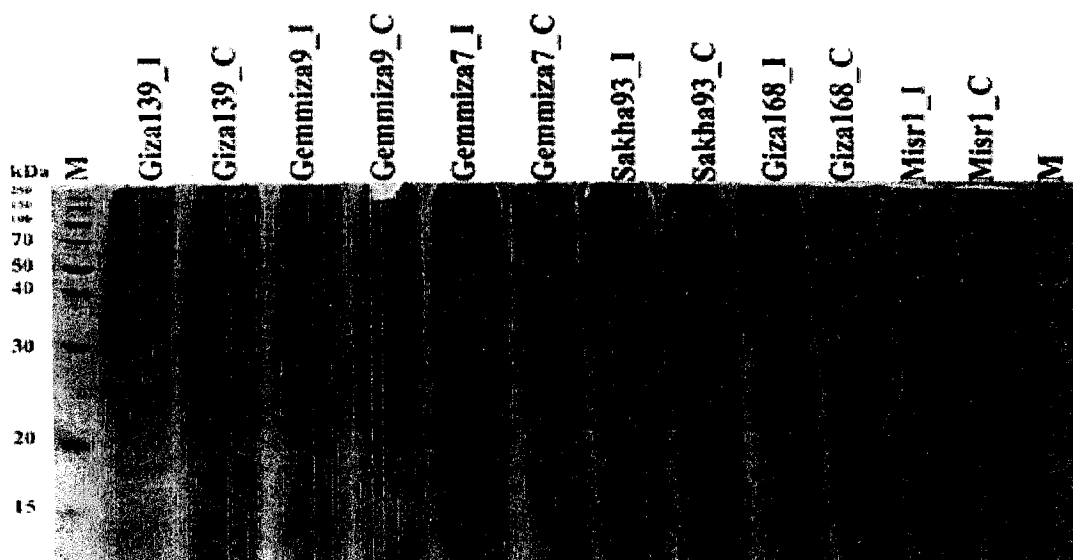


Figure (3): leaf total protein pattern of twelve leaf rust infected and non- infected wheat (*Triticum aestivum* L.) cultivars separated on 15% SDS-PAGE. The black arrows show positive protein marker. I: refers to infected cultivar while C: refers to the control cultivar.

For the total leaf protein pattern, some bands have been observed in the pattern of the rust resistance cultivar "Misr1" along with the other cultivars which they could be partially resistant to the leaf rust disease ("Giza-168" and "Sakha-93"). For example we can note such bands at molecular weight at about 17 kDa and 12 kDa, respectively in the pattern of the above mentioned cultivars (Figure 2).

The results of cluster analysis of protein data showed that the studied wheat cultivars were divided into three clusters. The first cluster consists of "Giza139_I", "Gemmiza-7_C", "Giza-168_I" and "Gemmiza-9_I" cultivars (Figure 4). The second cluster consists of "Giza-139_C", "Sakha-93_C", "Giza-168_C", "Sakha-93_I" and "Gemmiza-9_C" cultivars. The third cluster consists of "Misr-1_I" and "Misr-1_C" cultivars (Figure 4).

Thus, it seems that "Misr-1" cultivar is rust resistant cultivar according to the both pathological and biochemical experiments in comparing with the other cultivars. There are

contiguous in the results of both experiments (pathological and biochemical experiments) whereas they revealed that "Misr-1" cultivar is completely rust resistant wheat cultivar. This note appears from the cluster analysis, whereas "Misr-1" isolated in separated cluster apart of the other cultivars and this means that its protein structure does not significantly changed or affected with the rust infection. The other cultivars could be classified as either moderately rust resistant or rust susceptible cultivars because their protein structure has been affected by the rust infection. Dilara *et al* (2013) and Shaista *et al* (2011) studied the wheat biochemical protein pattern and they found differences in response to the rust disease among the studied wheat cultivars. So our results are in agreement with their results. Also, Nisar *et al* (2011) separated the rust resistance wheat cultivars using the SDS-PAGE technique from the Pakistan's wheat cultivars. Abdellatif *et al* (2012) found the same results in study of faba bean tolerance to drought stress.

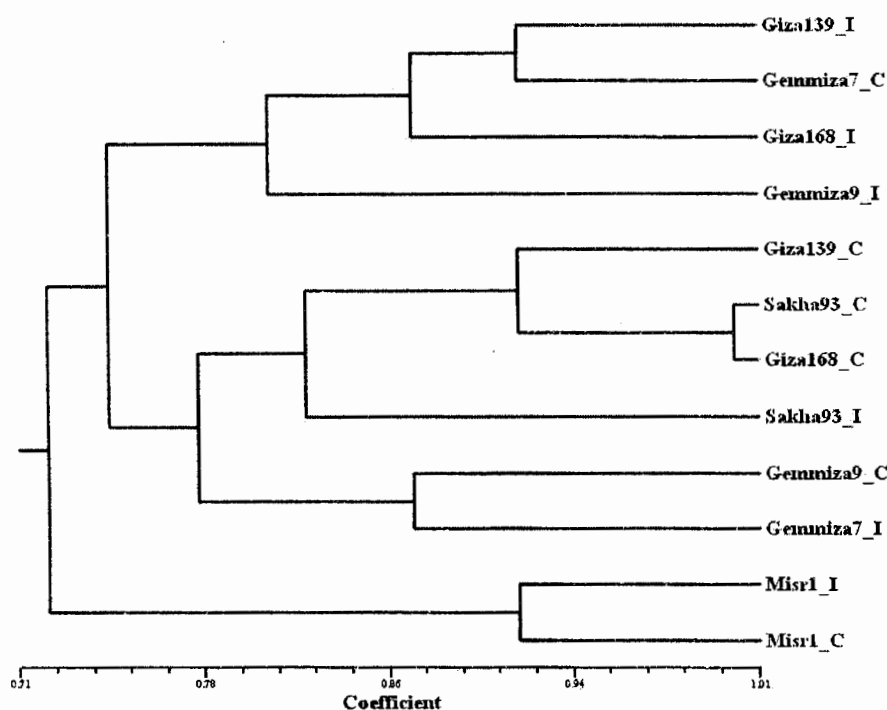


Figure (4): Phylogenetic dendrogram of of twelve leaf rust infected and non- infected wheat (*Triticum aestivum* L.) cultivars constructed depending upon biochemical markers using simple matching similarity coefficient and UPGMA method. I: refers to infected cultivar while C: refers to the control cultivar.

Thus it can be said that biochemical and pathological experiments revealed that "Misr1" wheat cultivar is rust resistance cultivar. Both pathological and biochemical results are almost the same and agreed with the most previous results. It can be also said that biochemical markers could be used to screen whether a wheat cultivar is rust resistant or not because some protein bands have been identified correlated with rust resistant. Thus biochemical markers can be used to save our effort and time to determine the rust resistant and susceptible wheat cultivars.

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التوصيف الكيموحيوي والمرضي لمقاومة صدأ الأوراق في بعض اصناف القمح المصرية

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الملخص العربي

الفطر بكسينيا ترينيسينا والمعرف علميا في الماضي بأسم بكسينيا ريكونداتا هو المسبب لمرض صدأ الأوراق في القمح ، والذي يعتبر من أهم وأخطر الأمراض الفطرية التي تصيب نبات القمح في مصر ، وقد استخدمت في هذه الدراسة ستة أصناف من القمح المصري Giza139، Giza168، Gemmiza7، Gemmiza9، Misr1 و Sakha93 لدراسة التوصيف المرضي والكيموحيوي لمقاومة الصدأ وقد تم فحص واختبار شتلات الأصناف

الستة من القمح في طور البادرة وكذلك استجابة النباتات البالغة للسلالة PTSS ، وأجري هذا الفحص لإختبار نوع العدوى، وشدة الإصابة النهائية وكذلك إختبار المساحة تحت المنحنى المرضي . أشارت النتائج إلى أن الصنف مصر- 1 تميز بصفة المقاومة الكاملة لمرض لصدأ الأوراق خلال طوري البادرة والنبات البالغ ، بينما الأصناف جيزة 168 و Gemmiza-9 سجلت قيم منخفضة للقياسات المرضية محل الدراسة ، بينما سجلت الأصناف جيزة 139 و Gemmiza-7 قيم مرتفعة . وقد لوحظت بعض حزم البروتين الذي يرتبط مع مقاومة الصدأ في القمح وكانت ذات وزن جزيئي حوالي 17 كيلو دالتون و ١٢ كيلو دالتون في نمط البروتين الكلي المنعزل من الأوراق، وعند حوالي 140 كيلو دالتون و ٣٤ كيلو دالتون لنمط البروتينات القابلة للذوبان في الماء (ناتجة أساسا في نمط بروتين الصنف "Misr1") المقاوم للصدأ تماما وفقا للتوصيف المرضي. وهكذا يمكن القول أن نتائج التجارب الكيموحيوية والتجارب المرضية اكدت أن الصنف "Misr1" مقاوم للصدأ. ويمكن القول أيضا أن الدلائل الكيموحيوية يمكن أن تستخدم لفحص ما إذا كان صنف القمح مقاومة للصدأ أو لا لأنه قد تم تحديد بعض حزم البروتين المرتبطة بالمقاومة للصدأ. وبالتالي توفر الجهد والوقت لتحديد أصناف القمح المقاومة للصدأ.