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EVALUATION OF Lr13 AND Lr24 GENES IN RESISTANCE TO LEAF RUST IN SOME EGYPTIAN WHEAT CULTIVARS

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ABSTRACT

In this study, each of the Egyptian wheat cvs., Sakha-61, Sakha-93, Sids-1, Giza-164 and Gemmeiza-3, susceptible to leaf rust caused by *Puccinia recondita f. sp. tritici* (race 77 under greenhouse and mixture of races 77 and 57 under field conditions), was crossed with one of the monogenic lines carrying either the leaf rust resistance Lr13 or Lr24 genes. The F1 populations in all crosses reacted against the leaf rust infection either at seedling or adult stages in similar way as the reaction of the resistant "donor" monogenic parent. Thus, the resistance to leaf rust at both seedling and adult stages is dominant over susceptibility in all tested F1 crosses. Exploration of protein bands using the SDS-PAGE technique emphasized the dominance of resistance over susceptibility in the F1 population of Lr24 x Sids-1 hybrid. The role of protein bands in manifestation of the resistant Lr24 gene was discussed.

As for the F2 generation, the plant populations were segregated and distributed along wide spectra of infection types (ITs 0 to 4) and disease severity (0 to 90S) at seedling and adult stages, respectively. Percentages of resistant reactions at seedling stage (ITs 0, 0;, 1 and 2) were ranged between 60.42-80.79% and 72.69-81.04% whereas, at the adult stage (DS 0 to 10MR) it was ranged between 71.94 - 75.97% and 70.0 – 81.28% for F2 populations of hybrids Lr13 x testers and Lr24 x testers, respectively. The highest percentages of resistant seedlings were recorded in F2 populations of Sids-1 crossed with the Lr13 and Lr24 monogenic lines, respectively. At the adult stage, the expression of Lr24 gene was better than Lr13 gene when introduced, for example, into Giza-164 (81.28 & 75.97%) and Gemmeiza-3 (80.33 & 71.94%). At both stages, the F2 plants of all crosses were segregated into resistant and susceptible phenotypes at ratios good fit to a ratio 3: 1, which indicated that the resistance to leaf rust disease is controlled by one pair of genes.

Conferring leaf rust resistance genes to commercial wheat cvs had a good effect on tillers formation, spike length and weight of 1000-kernels in F1 and F2 progenies compared with the receptor wheat cv.

Key words: *Puccinia recondita, leaf rust, resistance genes, Lr13, Lr24, monogenic lines.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important cereal crop in Egypt and all over the world. Among diseases affecting wheat plants, the leaf rust disease (*Puccinia recondita f. sp. tritici*) is one of the most serious and familiar rust diseases since, it caused severe losses in grain yield yearly (Roelfs *et al.* 1992). Losses due to this rust disease reached 23% on some wheat cvs., in Egypt (Nazim *et al.* 1984).

Resistant cultivars are considered the most economic tool for controlling rust diseases in several field crops. As for wheat leaf rust, many specific resistance genes are known and some of them have been introduced into wheat cultivars from wild relatives. It seems that when different leaf rust resistance genes are combined in the same line, the disease is more easily controlled: For example, Lr16 and Lr13 or Lr9 and Lr24 have been shown to guarantee reliable control (Long *et al.* 1993). In France and several European countries, no virulence has yet been detected for the Lr24 resistance gene, therefore its presence in combination with other resistance genes is desirable in European cultivars (Dedryver, 1996). Ezzahiri & Roelfs (1989) attributed the adult plant resistance to Lr13, Lr34 and a third, unknown genes, Lr34 which interacted in a complementary way to enhance resistance. Thakare, *et al.* (2001) mentioned that the Lr9, Lr19, and Lr24, were effective against eight pathotypes of *Puccinia recondita f.sp. tritici* prevailing at different localities in India, during rabi 1998-99. Also, the effectiveness of Lr16 genes conferring resistance against 757 isolates of wheat leaf rust pathogen (*P. recondita f.sp. tritici*) was monitored in the peninsular zone of India during 1995-99. Leaf rust resistance genes Lr9, Lr19 and Lr24 were not overcome by any virulent strains of the pathogen (Hasabnis, *et al.* 2002). Hasabnis, & Srikant Kulkarni (2002) detected the Lr resistance genes in two groups of wheat genotypes using the infection type matching technique. The Lr10, Lr13, Lr23, Lr26, and Lr34 were found either singly or in combination in 14 commercially grown wheat cultivars in India during 1996-97.

This study aimed to investigate if the introducing Lr13 and Lr24 genes could confer leaf rust resistance to some local wheat cultivars at the adult growth stage in Egypt.

MATERIALS AND METHODS

Breeding against wheat leaf rust:

Five wheat cultivars i.e. Sakha-61, Sakha–93, Sids-1, Giza-164 and Gemmeiza-3 varied in susceptibility to leaf rust infection and two monogenic lines (Lr13 and Lr24) exhibited a high resistant reaction against leaf rust were used in this study which was carried out at Sakha Agric. Research Station. In 1999/00 growing season all parents were sown in experimental plots 6 rows per each. Each of the later two monogenic lines was crossed as donor resistant parent (P1) with any the first five cultivars as susceptible parents (P2) to produce the seeds of the F_1 hybrids. In 2000/01 growing season, some of the resultant seeds of

the ten F1 hybrids were sown and self pollinated to produce the F_2 seeds while some others were stored to be used in the next season.

Screening for resistance to leaf rust infection at seedling stage:

In the season 2001/02, seeds of parents (P1 and P2) and the resultant hybrids (F1 and F2) were planted in 25 ϕ clay pots and kept in a conditioned greenhouse at Sakha Agric. Research Station. Seedlings 7-10 days old were uniformly inoculated with urediospores of the race 77, of leaf rust (*Puccinia recondita f. sp. tritici*) under Egyptian conditions as recovered by Abu El-Naga, *et al.* (1999) using the rubbing technique described by Stakman *et al.* (1962).

Table (1): Wheat leaf rust infection types scale used in disease assessment at seedling stage, (Johnston 1961).

Host response	Infection type	Disease description
Immune	0	No uredinia or other macroscopic sign of infection
Nearly immune	0;	No uredinia, but hypersensitive neurotic or chlorotic flecks present
Very resistant	1	Small uredinia surrounded by necrosis
Moderately resistant	2	Small to medium uredinia surrounded by chlorosis or necrosis
Moderately susceptible	3	Medium-sized uredinia that may be associated with chlorosis
Susceptible	4	Large uredinia without chlorosis

Urediospores of *Puccinia recondita*-race 77, the most aggressive and dominant race, were previously propagated on the susceptible wheat cv. Giza-139. Pots were kept for 48 hr under humid conditions then, transferred to the greenhouse at 20°C ±2 as adopted by Stubbs, (1988). To study the inheritance of leaf rust resistance at seedling stage, the rust infection type was recorded approximately 15 days from inoculation using the 0-4 scale (Table, 1) suggested by Johnston (1961). Seedlings exhibited infection types 0, 0;, 1 and 2 were considered resistant, while those of infection types 3 and 4 were considered as susceptible.

Screening resistance to leaf rust infection at the adult stage:

This experiment was conducted during the growing season 2001/02 in open field at Sakha Agric. Res. Sta. The experiment was performed in a randomized complete design with three replicates. Each replicate contained either 2 rows (each 3.5 m long and 30 cm apart) for each parent and F1 or 10 rows for each F2 population. The seeds were planted at 10 cm in-between (35 plant/row). For providing sufficient disease incidence, a mixture of highly susceptible wheat cvs (Giza-139, Sids-1 and Little club) were sown around the whole experiment as spreader to facilitate dissemination of urediospores of the leaf rust pathogen (*P. recondita f.sp. tritici*). The spreader wheat plants were moistened and dusted with spore powder mixture comprising the most prevalent leaf rust races 77 and 57 in

the experimental area plus talcum powder at the rate of 1:25 (w/w) as suggested by Tervet & Cassell (1951). All regular cultural practices were applied during the growing season. Disease severity and infection type (IT) of leaf rust were recorded 20 days after inoculation on each individual plant. Plants with disease severity of 0, 5R, 10R, 5MR and 10 MR considered resistant phenotypes, while, those recording 5MS, 10 MS, 10S, 20S, 40S and 80S were described as susceptible phenotypes (Peterson *et al.*, 1948). Then ratios of resistant to susceptible plants were determined. Statistical analysis were computed for all populations by determining the expected ratio and confirmed by X^2 analysis according to Steel and Torrie, (1960). Effects of conferring the leaf rust resistant genes Lr13 and Lr24 on the number of tillers/plant, spike length and 1000-kernel weight (g) were also recorded for each parent and F1 and F2 hybrids.

Protein electrophoresis

Samples of healthy wheat leaves were taken from 10-days old seedlings of P1 (Lr24), P2 (Sids-1), F1 and F2 to extract the water-soluble and non-soluble proteins according to Laemmli (1970). The electrophoresis was performed at room temperature in a vertical slab mold (16.5 x 14.5 x 0.1 cm and then the resulted gel is silver stained. Electrophoretic protein patterns of the tested samples were clustered by the average linked technique (un-weighed pair group method) Joseph *et al.* (1992). The results were expressed as phenograms. Cluster analysis was performed with computerized program.

RESULTS

Screening for resistance against leaf rust at seedling stage:

Data in Table (2) reveal that the monogenic parents i.e. Lr13 and Lr24 lines had low infection type (ITs) reactions (0; and 1 respectively) while, the wheat cvs., Sakha-61, Sakha-93, Sids-1, Giza-164 and Gemmeiza-3 had high infection types (3) against *P. recondita* f. sp. *tritici* (race 77) at seedling stage. Moreover, populations of the F1 hybrids exhibited resistant ITs (0;) for Lr13 x Sakha-61and Lr13 x Sakha-93 and (1) for Lr13 x Sids-1, Lr13 x Giza-164 and Lr13 x Gemmeiza-3. Similarly, F1 populations of Lr24 x Sakha-61, Lr24 x Sakha-93, Lr24 x Giza-164 and Lr24 x Gemmeiza-3 showed "0; ITs" whereas, Lr24 x Sids-1 exhibited "2 ITs". These results show that the character of resistance to leaf rust is dominant over susceptibility in all tested F1 crosses.

The data in Tables (2 & 3) prove also that, the F2 populations of a known tested cross (lines Lr13 and Lr24 x testers) were segregated and distributed along wide spectra of ITs ranged between 0-4. Resistant ITs (0, 0;, 1 and 2), however, were recorded on 78.26, 60.42, 80.79, 78.00 and 78.07% of the F2 populations of Lr13 x Sakha-61, Lr13 x Sakha-93, Lr13 x Sids-1, Lr13 x Giza-164 and Lr13 x Gemmeiza-3, respectively. The same resistant ITs (0, 0;, 1 and 2) were recorded also in 75.26, 74.42, 81.04, 72.69 and 74.65% of the F2 populations of Lr24 x Sakha-61, Lr24 x Sakha-93, Lr24 x Sids-1, Lr24 x Giza-164 and Lr24 x Gemmeiza-3, respectively.

The obtained results indicated that the Lr13 gene might be introduced into the tested commercial wheat cultivars through hybridization process easier than the Lr24 gene. The F2 populations resulted from crossing the commercial wheat cv. Sids-1 with the Lr13 and Lr24 monogenic lines produced the highest percentage of resistant plants i.e. 80.79 and 81.04%, respectively. Moreover, populations of the F2 plants resulted from crosses between the tow monogenic lines and these five commercial wheat cultivars were segregated into two phenotypes i.e. resistant and susceptible. The F2 populations were segregated at rates of 180:50, 145:95, 164:39, 156:44 and 178:50 for crosses with the monogenic Lr13 and 143:47, 160:55, 171:40, 173:65 and 162:55 for crosses with the monogenic Lr24. The segregation of F2 plants from the crosses of Lr13 and Lr24 x testers gave a good fit to the ratio of 3 resistant: 1 susceptible in both groups, which indicate the presence of one pair genes for resistance in this stage.

Table (2): Effect of introducing the Lr13 and Lr24 genes into some commercial wheat cultivars on the leaf rust (*Puccinia recondita* f. sp. *tritici*) infection type at the seedling stage.

Tested population	Examined plants No.	Infection types					
		Resistant				Susceptible	
		0	0;	1	2	3	4
P2 (Sakha-61)	48					48	
P2 (Sakha-93)	60					60	
P2 (Sids-1)	70					70	
P2 (Giza-164)	60					60	
P2 (Gemmeiza-3)	45					45	
P1 (Monogenic line Lr13)	50		50				
F1 (Lr13 x Sakha-61)	60		60				
F1 (Lr13 x Sakha-93)	50		50				
F1 (Lr13 x Sids-1)	55			55			
F1 (Lr13 x Giza-164)	50			50			
F1 (Lr13 x Gemmeiza-3)	67			67			
F2 (Lr13 x Sakha-61)	230	65	65	50		30	20
F2 (Lr13 x Sakha-93)	240	60	40	45		40	55
F2 (Lr13 x Sids-1)	203	63	55	25	21	15	24
F2 (Lr13 x Giza-164)	200	54	50	25	27	24	20
F2 (Lr13 x Gemmeiza-3)	228	50	55	30	43	40	10
P1 (Monogenic line Lr24)	45			45			
F1 (Lr24 x Sakha-61)	50		50				
F1 (Lr24 x Sakha-93)	35		35				
F1 (Lr24 x Sids-1)	43				43		
F1 (Lr24 x Giza-164)	38		38				
F1 (Lr24 x Gemmeiza-3)	46		46				
F2 (Lr24 x Sakha-61)	190	45	35	48	15	17	30
F2 (Lr24 x Sakha-93)	215	50	60	35	15	50	5
F2 (Lr24 x Sids-1)	211	61	50	38	22	15	25
F2 (Lr24 x Giza-164)	238	48	50	35	40	25	40
F2 (Lr24 x Gemmeiza-3)	217	39	76	35	12	55	

Table (3): Segregation (phenotypes and ratios) at seedling stage of the F2 populations of Lr13 and Lr24 crosses artificially inoculated with the leaf rust agent under greenhouse conditions.

F2 hybrid	Number of seedlings	Segregated Phenotypes				Expected ratio	X^2
		R		S			
		No.	%	No.	%		
Lr13 x Sakha-61	230	180	78.26	50	21.74	3:1	1.29
Lr13 x Sakha-93	240	145	60.42	95	39.58	3:1	0.54
Lr13 x Sids-1	203	164	80.79	39	19.21	3:1	0.81
Lr13 x Giza-164	200	156	78.00	44	22.00	3:1	0.95
Lr13 x Gemmeiza-3	228	178	78.07	50	21.93	3:1	1.31
Lr24 x Sakha-61	190	143	75.26	47	24.74	3:1	0.0
Lr24 x Sakha-93	215	160	74.42	55	25.58	3:1	0.02
Lr24 x Sids-1	211	171	81.04	40	18.96	3:1	4.08
Lr24 x Giza-164	238	173	72.69	65	27.31	3:1	0.67
Lr24 x Gemmeiza-3	217	162	74.65	55	25.35	3:1	0.13

Similarity and diversity of Lr24 and its crosses at protein level:

Data in Table (4), declared that the protein bands shown in the SDS-PAGE (Fig., 1) were greatly varied in their molecular weight (MW) and degree of staining. In addition to the faint appearance of the 52Kd protein band in the donor parent "Lr24" compared with the receptor "Sids-1" one, the receptor had specific 31, 28, 26 and 21Kd protein bands while, the donor characterized with bands of MW 37, 34 and 20Kd.

Table (4): Effect of introducing Lr24 gene into the commercial wheat cv. Sids-1 on the molecular weights of the separated protein bands in the F1 and F2 individual plants at seedling stage.

Peak number	Molecular weight of the protein band (Kd) for:					
	Sids-1	Lr24	F1	F2 individuals		
				No. 1	No. 2	No. 3
1	52	52	39	50	50	43
2	39	39	35	44	44	39
3	35	37	34	40	40	35
4	31	35	28	36	36	34
5	28	34	26	34	35	27
6	26	23	23	29	29	23
7	23	20	21	27	28	21
8	21	17	17	24	24	17
9	17	14	14	21	22	14
10	14	12	12	17	17	12
11	12	12	12	14	15	-
12	-	-	-	12	12	-

The observed variations in the characterized protein bands seemed to be responsible and positively correlated with degree of resistance against leaf rust. These facts were clearly shown in Fig. (2). In this figure, the receptor parent was

located in separate main cluster while the donor parent, F1 and F2 progenies were located in another separate main cluster with similarity of 88.49% between both main clusters. On the other hand the donor parent and the F1 progeny were located in single sub-cluster with 96.24% similarity in between. However, 2 out of the 3 individuals selected from the F2 progeny were located in sub-sub-cluster closely similar (98.19% similarity) while the third one was located in separate sub-cluster with average similarity 91.9% between them.

Screening for resistance against leaf rust at the adult stage:

Data in Table (5) show that, both monogenic lines Lr13 and Lr24 exhibited resistant reaction against infection with leaf rust races 77 and 57 at the adult stage under the open field conditions. In this regard, the monogenic line Lr24 recorded lower disease severity (10R) than the monogenic Lr13 (10MR). On the contrary, all tested wheat cultivars had high disease severity (DS) at the same stage. In this respect, Sids-1 recorded the highest DS (90S) followed by Sakha-61 and Sakha-93 (40S), Giza-164 and Gemmeiza-3 (30S), respectively.

All plants of the F1 hybrids (lines Lr13 and Lr24 x testers) showed similar resistant reaction particularly in testers crossed with the Lr24 monogenic line (10R). While, the F1 hybrids Lr13 x Sakha-61, Lr13 x Giza-164 and Lr13 x Gemmeiza-3 resist infection with leaf rust at the adult stage (10R) better than the donor monogenic Lr13 line (10MR).

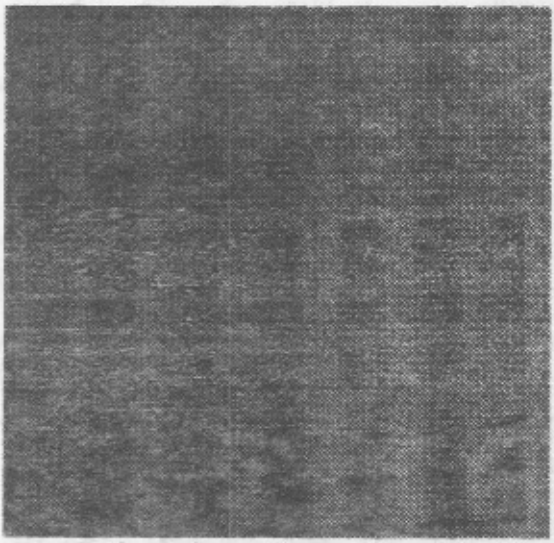

Fig. (1): SDS-PAGE for protein bands of the parents Sids-1 "Lane-1", Lr24 "Lane-2", F1 (lane-3) and the 3 individuals of F2 (Lanes 4, 5 and 6) at seedling stage.

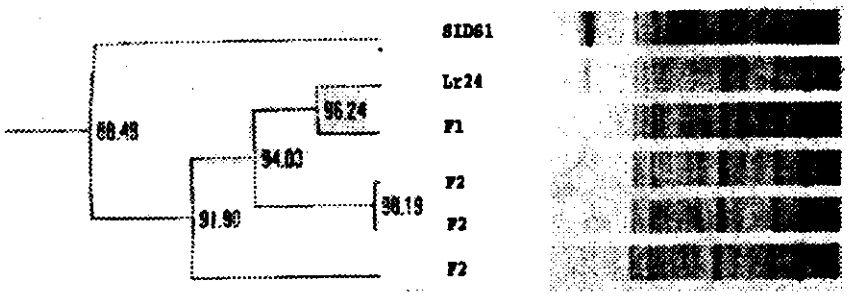

Fig. (2): Phenogram cluster analysis of protein patterns of P1 (Lr24), P2 (Sids-1), F1 (Lr24x Sids-1) and F2 (Lr24x Sids-1) wheat leaves at seedling stage.

Table (5): Effect of introducing the Lr13 and Lr24 genes into some commercial wheat cultivars on the leaf rust (*Puccinia recondita* f. sp. *tritici*) infection type at adult stage.

Tested Parents and Crosses	Examined Plants No.	Rust severity								
		Resistant classes			Susceptible classes					
		0	10 R	10 MR	10 MS	10 S	20 S	30 S	40 S	90 S
P2 (Sakha-61)	60								60	
P2 (Sakha-93)	45								45	
P2 (Sids-1)	49									49
P2 (Giza-164)	60							60		
P2 (Gemmeiza-3)	50							50		
P1 (Lr13)	60			60						
F1(Lr13 x Sakha-61)	55		55							
F1(Lr13 x Sakha-93)	48			48						
F1(Lr13 x Sids-1)	47			47						
F1(Lr13 x Giza-164)	58		58							
F1(Lr13 x Gemmeiza-3)	56		56							
F2(Lr13 x Sakha-61)	230	35	78	55	10	22	10	20		
F2(Lr13 x Sakha-93)	198	28	70	50	10		20		20	
F2 (Lr13 x Sids-1)	226	36	80	50	15	10	20		15	
F2(Lr13 x Giza-164)	233	47	70	60	21	10	15	10		
F2(Lr13 x Gemmeiza-3)	196	26	40	75	35		10		10	
P1 (Lr24)	50		50							
F1 (Lr24 x Sakha-61)	53		53							
F1 (Lr24 x Sakha-93)	50		50							
F1 (Lr24 x Sids-1)	62		62							
F1 (Lr24 x Giza-164)	57		57							
F1 (Lr24 x Gemmeiza-3)	62		62							
F2 (Lr24 x Sakha-61)	236	37	67	82	8	7	15	20		
F2 (Lr24 x Sakha-93)	240	25	80	63	25		26	21		
F2 (Lr24 x Sids-1)	251	38	96	51	15	26		25		
F2 (Lr24 x Giza-164)	219	38	78	62	20	10		11		
F2 (Lr24 x Gemmeiza-3)	239	45	66	81	10		20	17		

However, plants of the F2 generation (Tables, 5 & 6) showed wide range of disease severity ranged between resistant reactions (0 to 10MR) to the susceptible ones (40S to 90S). It is interest to state that some F2 plants (Table, 6) exhibited lower disease severity at the adult stage than the donor monogenic resistant lines. In this regard, 15.22, 14.14, 15.93, 20.17 and 13.27% of the F2 plants resulted from crossing each of Sakha-61, Sakha-93, Sids-1, Giza-164 and Gemmeiza-3 with the monogenic Lr13 recorded the highest resistant class (0) compared with the donor Lr13 which recorded the third resistant class (10MR). Similarly, the F2 plants resulted from crossing the same wheat cultivars with the monogenic Lr24 gave 15.68, 10.42, 15.14, 17.35 and 18.83% resistant class (0) compared with the Lr24 itself (10R). Percentage of plants showed different resistant classes (0 to 10MR), in general, were ranged between 71.94 - 75.97% and 70.0 – 81.28% for F2 hybrids Lr13 x testers and Lr24 x testers, respectively.

Table (6): Segregation for leaf rust resistance under greenhouse conditions of Lr13 and Lr24 crosses at adult stage under field conditions.

F2 hybrid	Number of plants	Segregated Phenotypes				Expected ratio	X^2
		R		S			
		No.	%	No.	%		
Lr13 x Sakha-61	230	168	73.04	62	26.96	3:1	3.71
Lr13 x Sakha-93	198	148	74.75	50	25.25	3:1	0.16
Lr13 x Sids-1	226	166	73.45	60	26.55	3:1	0.38
Lr13 x Giza-164	233	177	75.97	56	24.03	3:1	0.114
Lr13 x Gemmeiza-3	196	141	71.94	55	28.06	3:1	0.97
Lr24 x Sakha-61	236	186	78.81	50	21.19	3:1	0.64
Lr24 x Sakha-93	240	168	70.00	72	30.00	3:1	5.2
Lr24 x Sids-1	251	185	73.71	66	26.29	3:1	1.1
Lr24 x Giza-164	219	178	81.28	41	18.72	3:1	2.15
Lr24 x Gemmeiza-3	239	192	80.33	47	19.67	3:1	8.69

The superiority of a known monogenic lines for conferring leaf rust resistant gene (donor) seems to be depended on "the receptor" wheat cv. Based on percentage of resistant classes in the F2 populations, the present results reveal that the expression of Lr24 gene was better than Lr13 gene when introduced, for example, into Giza-164 (81.28 & 75.97%) and Gemmeiza-3 (80.33 & 71.94%). Data in Table (6) indicate also that the F2 populations for all tested crosses were segregated to resistant and susceptible phenotypes at ratios good fit to a ratio of 3: 1 which confirm the presence of one pair genes for resistance in the adult stage.

Data in Table (7) indicate that conferring leaf rust resistance genes (Lr13 &Lr24) to commercial wheat cvs had a good effect on tillers formation, spike length and weight of 1000-kernels in F1 and F2 progenies compared with the receptor wheat cv. In this respect, all tested crosses carrying Lr13 formed more tillers number than their female parents. The highest increase in tillers number/plant was recorded in F1 and F2 crosses of Lr13 x Sakha-61, Lr13 x Sakha-93, Lr24 x Sakha-61, Lr24 x Sids-1 and Lr24 x Sakha-93. Introducing the leaf rust resistant genes conspicuously improved spike length particularly in the

F1 progenies and to less extent in the F2 progenies comparing with their female parents. Also, conferring the Lr13 and Lr24 genes to commercial wheat cvs increased 1000-kernel weight of F1 and F2 crosses. The highest increase was recorded with the crosses i.e. Lr13 x Sids-1, Lr13 x Sakha-93 and Lr13 x Sakha-61of F1 progenies but this increase was not clear in F2 for the same crosses. Also, the same trend was recorded with crosses having Lr24 where the highest increase was recorded with the crosses i.e. Lr24 x Sakha-93, Lr24 x Sids-1 and Lr24 x Sakha-61 at F1 and with Lr24 x Sakha-93 and Lr24 x Sids-1 at F2.

Table (7): Effect of introducing the leaf rust resistance Lr13 and Lr24 genes into some commercial wheat cultivars on some yield components in the F1 and F2 progenies under stress of infection with *Puccinia recondita* f. sp. *tritici*.

Tested parents and Crosses	Wheat growth characters		
	Tillers No. /Plant	Spike length (cm)	1000-kernel weight (g)
P2 (Sakha-61)	14.2	11.50	43.41
P2 (Sakha-93)	13.8	11.13	46.23
P2 (Sids-1)	13.0	13.00	45.50
P2 (Giza-164)	11.0	11.50	43.80
P2 (Gemmeiza-3)	12.0	11.50	43.83
P1 (Monogenic line Lr13)	17.7	12.81	44.02
F1 (Lr13 x Sakha-61)	19.4	12.65	46.63
F1 (Lr13 x Sakha-93)	18.4	13.03	48.19
F1 (Lr13 x Sids-1)	15.2	14.11	51.00
F1 (Lr13 x Giza-164)	14.6	12.90	45.60
F1 (Lr13 x Gemmeiza-3)	13.6	11.30	45.16
F2 (Lr13 x Sakha-61)	19.8	11.74	43.60
F2 (Lr13 x Sakha-93)	17.2	12.15	45.11
F2 (Lr13 x Sids-1)	13.2	12.25	44.50
F2 (Lr13 x Giza-164)	14.2	12.0	44.60
F2 (Lr13 x Gemmeiza-3)	13.2	11.16	44.80
P1 (Monogenic line Lr24)	15.0	10.50	40.80
F1 (Lr24 x Sakha-61)	18.0	12.50	44.18
F1 (Lr24 x Sakha-93)	16.0	12.50	48.80
F1 (Lr24 x Sids-1)	17.0	13.50	46.50
F1 (Lr24 x Giza-164)	14.0	12.50	43.12
F1 (Lr24 x Gemmeiza-3)	13.5	12.50	43.18
F2 (Lr24 x Sakha-61)	17.2	12.00	42.20
F2 (Lr24 x Sakha-93)	14.0	12.00	46.15
F2 (Lr24 x Sids-1)	15.0	13.50	44.21
F2 (Lr24 x Giza-164)	13.5	12.00	41.00
F2 (Lr24 x Gemmeiza-3)	13.0	11.90	41.18

DISCUSSION

Wheat (*Triticum aestivum* L.) is the most important cereal crop in Egypt and all over the world. Leaf rust (*Puccinia recondita f.sp. tritici*) is one of most important common foliar rust diseases attacked wheat plants causing huge losses yearly (Abd El-Hak *et al.* 1972, Bajwa *et al.* 1986 and Abu El-Naga *et al.* 1999). Since occurrence of stem rust in epidemic form on wheat in Egypt during 1945, a large number of wheat cultivars that had high production characters and resistant to rusts were produced through the breeding programs. Because appearance of more virulent or new rust races in addition to changes in climatic conditions in the last years, the resistance against leaf and stripe rusts had been broken on some of these cultivars although they still possessing highly productivity. In this respect, many successful attempts were made to introduce Lr genes (Dedryver, *et al.*, 1996), Yr genes (Stubbs, 1988) and Sr genes (Sawhney and Goel, 1986) from monogenic lines to susceptible wheat cvs. Thus, the present study aimed to investigate efficacy of Lr13 and Lr24 resistant genes that conferred to some commercial wheat cultivars from 2 monogenic lines particularly at the adult stage.

The obtained results indicated that the F1 populations acquired the leaf rust resistance genes (from monogenic lines) reacted against the leaf rust infection either at seedling or adult stages in similar way as the reaction of the resistant "donor" monogenic parent. Thus, the resistance to leaf rust at both seedling and adult stages is dominant over susceptibility in all tested F1 crosses. This observation was true in the F1 population of all performed crosses. The dominance of the leaf rust resistance genes over the susceptibility was confirmed by Barcellos *et al.*, (2000), and Kolmer and Liu, (2001) where they verified that Lrs genes such as Lr13, Lr16, Lr27, Lr34, Lr37 and Lr 46 governed the resistance of wheat cvs to leaf rust at adult and seedling stages.

Exploration of protein bands using the SDS-PAGE technique reveal that the degree of similarity was high between the donor of the resistance Lr24 gene and the F1 progeny resulted from Lr24 x Sids-1 (96.24%). Both donor parent and F1 progeny was located in single sub-cluster while, the receptor parent was located in another separate cluster. This finding might explain the dominance of the Lr24 gene over the susceptibility to leaf rust infection. Both donor and receptor parents as well as their F1 and F2 progenies were greatly varied in the molecular weight (MW) and degree of staining of the detected protein bands. These variations in the characterized protein bands seemed to be responsible and positively correlated with degree of resistance against leaf rust.

It was found that, the populations of any F2 generation were segregated and distributed along wide spectra of infection types (ITs 0 to 4) and disease severity (0 to 90S) at seedling and adult stages, respectively. Among the F2 populations, percentages of plants exhibited resistant reactions were ranged between 60.42-81.04% at seedling stage and 70.0 – 81.28% at the adult stage depending on kind of the source of Lr genes (Donor) and the used female commercial cultivar (Receptor). The genetic background of the receptor parent might be very important for manifestation of the introduced Lr genes. In point, the F2 populations of Sids-1 crossed with the Lr13 and Lr24 monogenic lines

produced the highest percentages of resistant seedlings. At the adult stage, the expression of Lr24 gene was better than Lr13 gene when introduced, for example, into Giza-164 (81.28 & 75.97%) and Gemmeiza-3 (80.33 & 71.94%). At both stages, the segregation ratio of the resistant to susceptible plants in the F2 populations of all crosses was good fit to a ratio 3: 1, which suggested that a single, dominant locus defined this resistance. These results are in agreement with Sawhney & Goel (1986) who stated that the near isogenic lines and cultivars of wheat having single leaf rust resistance genes (Lr2a, Lr19, Lr21, Lr22, Lr23, Lr24, Lr25, Lr26, Lr27 and LrKT) were found to be resistant also to the Indian stem rust. Kolmer, (1992) in North America, McIntosh, (1992) in Australia, and Sawhney *et al.* (1992) in India verified also that, Lr13 is highly effective at adult plant resistance against leaf rust and it may play an important role in interaction with other gene. The present results prove also that, conferring Lr13 & Lr24 genes to tested Egyptian commercial wheat cvs improved tillers forming and spike length as well as increased 1000-kernel weight of F1 and F2 progenies.

The obtained results could be discussed in light the concept gene for gene relationship of Flor, (1965) and the others that used this concept to make similar interactions between specific genes like Loegering & Powers (1962) for wheat stem rust, and Samborski & Dyck, (1968) for leaf rust. In this regard, similar results were obtained also by Samborski & Dyck, (1982), Singh & McIntosh (1984), Dyke, (1994) and Sawhney (1992) who found that the interactive gene conferring resistance in Chinese spring was later identified as Lr34. While McIntosh *et al.* (1977) indicated that Lr24 is tightly linked with stem rust resistance gene Sr24 and provides a good resistance against leaf and stem rust. Therefore, Ezzahiri & Roelfs, (1989), Kolmer, (1992) in North America, McIntosh, (1992) in Australia, and Sawhney *et al.* (1992) in India verified that Lr13 is highly effective at adult plant resistance against leaf rust and it may play an important role in interaction with other gene. Hasabnis, *et al.* (2002) verified that the prevailing dominant population of the fungus had lost virulence on Lr10, Lr13, Lr15, Lr20 and Lr23. The Lr genes viz., Lr9, Lr19, and Lr24 conformed resistance to the pathogenic isolates whereas Lr2c and Lr14 conformed susceptibility. Genes from the near-isogenic lines, Lr10, Lr20, Lr15, Lr13, Lr23 and Lr26 conformed resistance to the pathogen next to the intact genes Lr9, Lr 19, and Lr 24.

Finally, introducing Lr13 and Lr24 into susceptible highly producible wheat cvs in Egypt will be useful in providing these cvs with ensured resistance against prevalent leaf rust races. Also, this step could be serving in improving breeding programs for increasing productivity and resistance efficacy in one time for the three rusts attacking wheat under Egyptian conditions.

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تقييم جينات Lr13 وLr24 فى مقاومة صدأ الأوراق لبعض أصناف القمح المصرية

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فى هذه الدراسة، عندما هجن أى من أصناف القمح المصرية القابلة للإصابة بصدأ الأوراق الناتج عن فطر باكسينيا ركونديتا تريتيسای مسبب صدأ الأوراق (سلالة ٧٧ تحت ظروف الصوبة وخليط من السلالتين ٧٧، ٥٧ تحت ظروف الحقل) مثل سخا-٦١ ، سخا-٩٣ ، سدس-١ ، جيزة-١٦٤ ، جميزة-٣ مع أى من سلالات القمح أحادية الجين Lr13أو Lr24 كانت كل أفراد الجيل الأول لكل الهجن الناتجة مقاومة لعدوى صدأ الأوراق فى مرحلتى البادرة والبلوغ بطريقة مشابهة لسلوك سلالات القمح أحادية الجين المانحة. لذلك يمكن القول أن المقاومة لعدوى صدأ الأوراق فى مرحلتى البادرة والبلوغ سائدة على القابلية للإصابة فى كل هجن الجيل الأول المختبرة. وقد أكدت طريقة التفريد الكهربى للبروتين سيادة المقاومة على القابلية للإصابة فى أفراد الجيل الأول للهجين Lr24 x Sids-1. كما نوقشت روابط البروتين المتكونة فى إظهار دور جين المقاومة Lr24.

بالنسبة للجيل الثانى، إنعزلت النباتات على مدى واسع من الطرز المرضية للعدوى (ITs 0- 4) وشدة الإصابة (DS 0 - 90S) فى مرحلتى البادرة والبلوغ على التوالى، وقد تراوحت ردود الفعل المقاومة للعدوى (ITs 0, 0;, 1 and 2) فى مرحلة البادرة لتكون بين ٦٠,٤٢-٨٠,٧٩% و ٧٢,٦٩- ٨١,٠٤% فى حين تراوحت شدة الإصابة (DS 0 to 10MR) فى مرحلة البلوغ بين ٧١,٩٤- ٧٥,٩٧% و ٧٠,٠- ٨١,٢٨% لعشائر الجيل الثانى لهجن Lr13 x testers, Lr24 x testers على التوالى. وقد سجلت أعلى نسبة منوية للبادرات المقاومة لأفراد الجيل الثانى الناتجة من تهجين سدس-١ مع سلالات القمح أحادية الجين Lr13أ و Lr24 على التوالى. وفى مرحلة البلوغ، كان تأثير Lr24 أفضل من Lr13 عندما منح إلى جيزة-١٦٤ (٨١,٢٨ & ٧٥,٩٧%) وجميزة-٣ (٨٠,٣٣ & ٧١,٩٤%) على سبيل المثال. وفى كلا المرحلتين، إنعزلت نباتات كل هجن الجيل الثانى بنسبة ٣ مقاوم إلى ١ قابل للإصابة لكلا المجموعتين مشيرة إلى أن المقاومة لمرض صدأ الأوراق يحكمها وجود زوج واحد من الجينات. كما كان لمنح جينات المقاومة ضد صدأ الأوراق تأثير جيد على تكوين الأشطاء وطول السنبلة ووزن ألف حبة لنباتات الجيل الأول والثانى مقارنة بأصناف القمح الأم.