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Inheritance and Detection of Leaf Rust Resistance Genes in Some Egyptian Wheat Cultivars

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Abstract: Resistance to leaf rust caused by *Puccinia triticina* can be improved in wheat cultivars by detection of leaf rust resistance genes that are present in the wheat cultivars. This study was carried out during 2016/17, 2017/18 and 2018/19 growing seasons to study the inheritance and genetic nature of wheat leaf rust resistance in eight Egyptian bread wheat cultivars *i.e.* Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Sids 1, Sids 12, Sids 13 and Sids 14. Crosses were done between the eight tested wheat cultivars and the highly susceptible wheat variety; Thatcher to determine the nature of leaf rust resistance and number of leaf rust resistance genes in these cultivars. Also, crosses were done between the tested wheat cultivars and six leaf rust monogenic lines *i.e.* *Lr* 50, *Lr* 51, *Lr* 54, *Lr* 64, *Lr* 67 and *Lr* 68 to determine the presence of these genes in the tested cultivars. The F_1 seeds were planted to produce F_2 plants. The resulted F_2 plants were tested at adult plant stage at Behira governorate during 2018/19 growing season. Segregations of F_2 plants at adult plant stages indicated that the wheat cultivar Gemmeiza 12 has the two leaf rust resistance genes; *Lr* 50 and *Lr* 67 and the wheat cultivar Sids 14 has one leaf rust resistance gene; *Lr* 64. While, the other tested wheat cultivars doesn't carry any of the tested leaf rust resistance genes at adult-plant stage. We recommend using these leaf rust resistance genes in the breeding program to improve wheat resistance to leaf rust.

Keywords: Wheat, Leaf Rust, *Puccinia triticina*, Resistance Genes

1. Introduction

Wheat (*Triticum aestivum* L.) is considered one of the most important cereal crops in Egypt as well as in many parts of the world. Wheat plants are suffering from many destructive diseases. Rusts are the most important diseases of wheat because of their ability to move for a long distance and their ability to form new virulent races causing serious losses [1-5]. Leaf rust caused by *Puccinia triticina* Eriks. is a widespread disease in wheat in Egypt and worldwide causing significant losses in grain yield. Susceptible wheat cultivars to leaf rust suffer from yield reduction between 5 to 60% [6].

The occurrence of severe and damaging epidemics of leaf rust caused many new wheat cultivars to be eliminated and discarded very shortly after their release and farmer's use in agriculture. In Egypt significant losses grain yield exceeded to 23% in the susceptible wheat cultivars under suitable environmental conditions particularly in the north parts of Delta [7-11]. Breeding for resistance is far more cost effective and environmentally friendly than through fungicide application [1, 12]. Although host-genetic resistance to rust disease has commonly provided to acceptable protection without the need for chemicals [13]. Quantitative resistance that delays the epidemic development of leaf rust in wheat is an important source for durable resistance, quantitative resistance is often more durable than qualitative resistance [14, 15]. The strategy of wheat breeder and pathologists has always been concentrating on adult plant resistance genes (APR) in order to identify and improve the level of resistance [16] and aims to increase wheat production through genetic improvement of wheat cultivars against rust diseases.

This study wish to update and review various aspects of genetic of leaf rust resistance in some Egyptian wheat cultivars and attempt to relate this genetic information with the effectiveness and durability of resistance.

The main objective of this study was to determine the type of resistance and number of leaf rust resistance genes in eight Egyptian wheat cultivars. Also, to inherit and detect six leaf rust resistance genes in the tested wheat cultivars through genetic analysis.

2. Materials and Methods

Eight Egyptian wheat cultivars, six leaf rust monogenic lines and the susceptible wheat variety Thatcher (Table 1) were used to determine the type of resistance, the number of gene (s) and which gene was present in the tested wheat cultivars. For the first time in Egypt, grains of the six leaf rust monogenic lines; *Lr* 50, *Lr* 51, *Lr* 54, *Lr* 64, *Lr* 67 and *Lr* 68 were provided to Egypt by International Maize and Wheat Improvement Center (CIMMYT), Mexico, through the website (http:// www.cimmyt.org/seed-request/#wheat).

Table 1. *Pedigree and year of release of the wheat genotypes under study.*

Wheat genotype	Pedigree	Year of release
a- Bread wheat cultivars:		
Sids 1 (check)	HD2172/PAVON"S"//1158.574"S". SD46-4SD-2SD-1SD-0SD.	1996
Gemmeiza 9	ALD"S"/HUAC"S"//CMH74A. 630/SX. GM4583-5GM-1GM-0GM.	1999
Gemmeiza 10	MAYA74"S"/0N//160-147/3/BB/GLL/4/CHAT"S"/5/CROW"S". GM5820-3GM-1GM-2GM-0GM.	2004
Sids 12	BUC//7C/ALD/5/MAYA74/ON//1160-147/3/BB/GLL/4/CHAT"S"/6/MAYA/VUL-4SD-1SD-1SD-0SD.	2007
Sids 13	KAUZ "S"//TSI/SNB"S". ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP-0SD.	2010
Sids 14	SW8488*2/ KUKUNACGSS01Y00081T-099M-099Y-099M-099B-9Y-0B-0SD.	2018
Gemmeiza 11	B0W"S"/KVZ"S"//7C/SERI82/3/GIZA168/SAKHA61. GM7892-2GM-1GM-2GM-1GM-0GM.	2011
Gemmeiza 12	OTUS/3/SARA/THB//VEE.CCMSS97Y00227S-5Y-010M-010Y-010M-2Y-1M-0Y-0GM	2017
b- Monogenic lines (*Lr*'s):		
Lr 50	*Triticum timopheevii rmeniacum*	2003
Lr 51	*Triticum speltoides*	2005
Lr 54	*Aegilops kotschyi*	2005
Lr 64	*Triticum dicoccoides*	2009
Lr 67	PI 250413	2010
Lr 68	Parula	2012
Thatcher	*Triticum aestivum*	1990

The experiments of this study were carried out under field conditions at Elbostan, Behira governorate (30°45'19.4"N, 30°29'04.8"E) during three successive growing seasons, *i.e.* 2016/17, 2017/18 and 2018/19. The parents genotypes and leaf rust monogenic lines were grown in plots, each contains 10 rows of 4 m long and 30 cm apart during 2016/17 growing season in three successive sowing dates at 15 days intervals to overcome differences in the time of flowering. The leaf rust monogenic lines were used as male parents for crosses with each of the tested wheat cultivar to obtain F_1 seeds. Also, each of the tested wheat cultivars was used as male parent for the crosses with the wheat variety; Thatcher. The F_1 seeds were grown in the following season; 2017/18 in rows of 4 m long and 30 cm apart in order to facilitate production of F_2 seeds. In 2018/19 growing season, parents, the rest of F_1 and F_2 seeds were grown at Elbostan location, Behira governorate in plots; each plot contains 13 rows of 4 m long and 30 cm between rows and seeds were 20 cm apart, therefore each row contained 20 plants and each plot contained 260 plants. Each of F_2 cross was evaluated at two plots contained 520 plants. All plots were surrounded by a spreader area in one meter width sown with a mixture of the highly susceptible wheat cultivars, *i.e.* Morocco and *Triticum spelta saharensis*.

Inoculation and disease assessment:

Spreader plants were sprayed with water and dusted with spores powder mixture of the most prevalent and aggressive leaf rust pathotypes *i.e.* FTSSS, KTSPT, NTTJT, NTTKT, NTTTT, PTTCT, PTTGS, PTTNS, STTTK and TTTBT [4] mixed with talcum powder, at a rate of one volume of fresh urediniospores to 20 volumes of talcum powder according to Roelfs *et al*. [17]. Dusting was carried out in the early evening (at sunset) before dew point formation. The inoculation of all plants was carried out at booting stage according to Tervet and Cassell [18]. Data of leaf rust severity were recorded at the adult plant stage of the tested plants using the modified Cobb's scale [19]. Plant reaction was expressed in five infection types [17]. The infection types were immune = (0), resistant = (R), moderately resistant = (MR), moderately susceptible = (MS) and susceptible = (S).

$$AUDPC = D\left[\frac{1}{2}(Y_1 + Y_k) + (Y_2 + Y_3 + \ldots\ldots + Y_{k-1})\right]$$

Where:

D = days between two consecutive records (time intervals)

$Y_1 + Y_k$ = Sum of the first and last disease scores.

$Y_2 + Y_3 + \ldots\ldots. + Y_{k-1}$ = Sum of all in between disease scores.

Percent rust severity was recorded based on the modified Cobb's scale [19] for parents and each of F_2 plant at milk stage when the susceptible wheat cultivars Morocco and *Triticum spelta saharensis* displayed a response between 80 S to 100 S. The F_2 plants of each cross were grouped into eight classes depending on their percentage of disease severity under field conditions. The disease severity classes were: 0-10; >10-20; >20-30; >30-40; >40 50; >50-60; >60-70 and >70-80. Plants grouped in the first three classes were considered as resistant phenotype, while plants of the other classes (more than 30%) were considered as susceptible phenotype [20].

For identification of the adult plant leaf rust resistance genes in each cross, goodness of fit of the observed to the expected ratio of the phenotypic classes concerning the leaf rust severity and infection types, were determined by Chi-square (χ^2) analysis according to Steel and Torrie [21]. Moreover, the minimum number of effective genes controlling slow-rusting resistance in each cross was estimated by the formula of Wright [22]. This formula assumes that, there is no linkage, no epitasis, no dominance, all loci have equal effects and all genes controlling resistance are in a single parent of the cross. Degrees of dominance were calculated according to Romero and Frey [23]. Heritability in its broad-sense was estimated according to Lush [24].

3. Results

Evaluation of the tested wheat genotypes against leaf rust under field conditions:

Final rust severity (FRS%): Large variations in final rust severity between the tested wheat genotypes ranged from 0 to 70% during the three growing seasons of the study at Elbostan location. The 14 tested wheat genotypes were classified into three categories based on the leaf rust response (rust severity (%) and infection type). The first category is varieties having race specific resistance which contained the wheat genotypes displayed immune and genotypes with infection type resistance (R) and moderately resistance (MR). This category contained the two wheat genotypes; *Lr* 51 and *Lr* 54 which displayed field response from 0 to 5 MR during the three growing seasons (Table 2).

The second category is genotypes having partial resistance (slow rusting resistance) which contained wheat genotypes with infection type susceptible (S) and rust severity to 30%. This category contained 11 wheat genotypes; Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Sids 12, Sids 13, Sids 14, *Lr* 50, *Lr* 64, *Lr* 67 and *Lr* 68, which showed field response ranged from Tr MS to 20 S at Elbostan location during the three seasons (Table 2).

The last category is fast rusting varieties which contained wheat variety displayed infection type susceptible (S) and rust severity above 30%. This category contained check variety; Sids 1 only which displayed field response from 50 S to 70 S at Elbostan location (Table 2).

Area under disease progress curve (AUDPC): The 14 tested wheat genotypes were classified into three categories based on AUDPC values. The first category is varieties having race specific resistance which contained the wheat varieties displayed the lowest AUDPC values. This category contained two wheat genotypes; *Lr* 51 and *Lr* 54 which displayed AUDPC values ranged from 0 to 49 during the three growing seasons (Table 3).

Table 2. *Final leaf rust severity (FRS %) and infection type (IT) of 14 wheat genotypes grown at Elbostan location during 2016/17, 2017/18 and 2018/19 growing seasons.*

Genotype	Season / FRS (%)		
	2016/17	2017/18	2018/19
Genotypes with race specific resistance			
Lr 51	0	0	Tr MR
Lr 54	Tr MR	5 MR	5 MR
Genotypes with partial resistance (slow rusting resistance)			
Gemmeiza 9	Tr S	Tr S	5 S
Gemmeiza 10	10 S	5 S	Tr S
Gemmeiza 11	20 S	20 S	10 S
Sids 12	5 S	5 S	Tr S
Sids 13	5 S	5 S	Tr S
Sids 14	Tr S	10 S	Tr S
Gemmeiza 12	5 S	5 S	Tr S
Lr 50	5 S	10 S	5 S
Lr 64	5 MS	Tr MS	Tr MS
Lr 67	Tr MS	Tr MS	Tr MS
Lr 68	5 MS	5 MS	5 MS
Fast rusting cultivar			
Sids 1 (check)	60 S	70 S	50 S

Table 3. *Area under disease progress curve (AUDPC) of 14 wheat genotypes grown at Elbostan location during 2016/17, 2017/18 and 2018/19 growing seasons.*

Genotype	Season / AUDPC		
	2016/17	2017/18	2018/19
Genotypes with race specific resistance			
Lr 51	0	0	42
Lr 54	42	49	49
Genotypes with partial resistance (slow rusting resistance)			
Gemmeiza 9	42	42	49
Gemmeiza 10	80.5	49	42
Gemmeiza 11	157.5	157.5	80.5
Sids 12	49	49	42
Sids 13	49	49	42
Sids 14	42	80.5	42
Gemmeiza 12	49	49	42
Lr 50	49	80.5	49
Lr 64	49	42	42
Lr 67	42	42	42
Lr 68	49	49	49
Fast rusting cultivar			
Sids 1 (check)	700	840	560

The second category is genotypes having partial resistance which displayed moderate AUDP values less than 300. This category contained 11 wheat genotypes; Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Sids 12, Sids 13, Sids 14, *Lr* 50, *Lr* 64, *Lr* 67 and *Lr* 68, which displayed AUDPC values ranged from 42 to 157.5 during the three seasons (Table 3).

The last category is fast rusting varieties which contained the check wheat variety; Sids 1 only that displayed high AUDP values (more than 300) ranged from 560 to 840 during the three seasons (Table 3).

To study the inheritance mode of leaf rust resistance at adult plant stage in eight bread wheat cultivars, *i.e.* Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Sids 1, Sids 12, Sids 13 and Sids 14 crosses were made among these cultivars and the highly susceptible wheat variety; Thatcher. However, the two parents, F_1 and F_2 populations for each cross were tested at the adult stage under field conditions in 2018/19 growing season at Elbostan location.

The qualitative analysis:

The qualitative analysis of the obtained data was carried out according to the response of the tested parents, F_1 and F_2 populations against wheat leaf rust pathogen at the adult plant stage under field conditions using a mixture of the available urediniospores of the pathogen *Puccinia triticina* races. Crosses between the eight cultivars and the highly susceptible wheat variety; Thatcher indicate that, F_2 plants having low: high leaf rust severity were 271: 29, 280: 20 and 275: 25 in the crosses between (Thatcher × Gemmeiza 9, Gemmeiza 12 and Sids 12). These segregations fitted the theoretical expected ratios of 15:1 in order with P. values 0.014, 0.766 and 0.136 respectively. These results confirmed that at least two independent dominant genes pairs are controlling leaf rust disease in each of the three crosses Gemmeiza 9, Gemmeiza 12 and Sids 12. While, one dominant gene was find in crosses (Thatcher × Gemmeiza 10, Sids 13 and Sids 14 the numbers of F_2 plants having low: high leaf rust severity were 218:82, 219:81 and 217:83, expected ratios were 3:1 in order with P. values 0.351, 0.424 and 0.286, respectively. On the other hand, numbers of F_2 plants having low: high leaf rust severity was 25: 275 in the cross Thatcher × Sids 1. These numbers fitted the theoretical expected ratios of 1:15 with P. values 0.136. These results confirm that at least two independents recessive genes pairs are controlling leaf rust in this cultivar. While, numbers of F_2 plants having low: high leaf rust severity was 7: 293 in the cross of Thatcher × Gemmeiza 11. These numbers fitted the theoretical expected ratios of 1:63 with P. values 0.282. These results confirm that at least three independents recessive genes pairs are controlling leaf rust in this cultivar (Table 4).

Table 4. *Segregation of F_2 plants of the crosses among Thatcher and each of eight wheat cultivars as well as their respective parents and F_1 inoculated with Puccinia triticina at the adult plant stage under field conditions in 2018/2019 growing season.*

No.	Cross name	No. of tested plants	Observed ratio		Expected ratio	Chi-Square value	
			L	H		χ^2	P^b
1	Thatcher × Gemmeiza 9	P1 50					
		P2 50					
		F_1 50					
		F_2 300	271	29	15:1	2.97	0.014
2	Thatcher × Gemmeiza 10	P1 50					
		P2 50					
		F_1 30					
		F_2 300	218	82	3:1	0.871	0.351
3	Thatcher × Gemmeiza 11	P1 50					
		P2 50					
		F_1 50					
		F_2 300	7	293	1:63	1.159	0.282
4	Thatcher × Gemmeiza 12	P1 50					
		P2 50					
		F_1 50					
		F_2 300	280	20	15:1	0.089	0.766
5	Thatcher × Sids 1	P1 50					
		P2 50					
		F_1 50					
		F_2 300	25	275	1:15	2.22	0.136
6	Thatcher × Sids 12	P1 50					
		P2 50					
		F_1 30					
		F_2 300	275	25	15:1	2.22	0.136
7	Thatcher × Sids 13	P1 50					
		P2 50					
		F_1 50					
		F_2 300	219	81	3:1	0.640	0.424
8	Thatcher × Sids 14	P1 50					
		P2 50					
		F_1 50					
		F_2 300	217	83	3:1	1.38	0.286

L = Low rust severity ≤ 30%; H = High rust severity > 30%; P^b = Values higher than 0.05 indicate no Significance of χ^2.

To study the genetic behavior of wheat leaf rust resistance quantitatively, the two parents, F_1 and F_2 populations for each of the eight crosses were tested at the adult plant stage under field conditions. Population means (X) and variances (S^2) of the parents, F_1, s and F_2, s were used to estimate the degrees of dominance for F_1 (h_1) and F_2 (h_2), the heritability in its broad–sense and the number of functioning genes for each crosses (Table 5).

Means and degrees of dominance:

The average means for the susceptible wheat leaf rusting second parent Thatcher and the other eight cultivars used, *i.e.* Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Sids 1, Sids 12, Sids 13 and Sids 14 were 74, 17, 29, 29, 6, 58, 10, 5.6 and 5.4 respectively, under the field conditions (Table 5). The obtained data proved that the leaf rust severity mean values of the F_1 plants in the eight crosses were 32.6, 38, 43, 18, 74, 18, 18 and 16 respectively, these means were lower in Gemmeiza 9, Gemmeiza10, Gemmeiza 11, Gemmeiza 12, and Sids 12 than their respective mid parent values, indicating the presence of partial dominance for resistance. On the other hand, cross (Thatcher × Sids 1) exhibited higher means of rust severity (74) higher than their respective mid parent values indicating the presence of over dominance for susceptibility in this cross. The F_2 means for these eight crosses were 17.13, 45.76, 45.46, 6.7, 41.5, 17.3, 18.6 and 10.06, respectively. The means which were lower than their respective mid parent values indicating the presence of partial dominance of resistance over susceptibility and confirming the results obtained from the F_1, s (Table 5), except crosses (Thatcher × Gemmeiza 10, Gemmeiza 11 and Sids 13), which were higher than their respective parents values have respective mid–parent values indicating the presence of over dominance toward susceptibility over resistance in these crosses. Expression of gene actions measured as the degree of dominance h_1 and h_2 are shown in (Table 5). The estimated values of h_1 were -0.45, -0.60, -0.37,-0.64, +1, -0.75, -0.63and -0.69 for the eight crosses with cultivars Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Sids 1, Sids 12, Sids 13 and Sids 14 in sequence. The significant negative values of h_1 revealed the presence of partial dominance for resistance. While the estimated values for degrees of dominance h_1 was +1 to crosses (Thatcher × Sids 1). The significant positive values of h_1 revealed the presence of complete dominance for susceptibility in this cross. The estimated values of degrees of dominance of F_2 (h_2) were -1.9, -3.14, -0.51, -1.95, -6.12, -1.54, -1.23 and -1.72 for the eight crosses with cultivars, Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Sids 1, Sids 12, Sids 13 and Sids 14 respectively. The negative values estimated in these crosses suggested the partial dominance for leaf rust resistance.

Expression of genes actions measured as the degree of dominance showed that the significant negative values of h1, h2 revealed the presence of partial dominance for resistance and the significant positive values of h1, h2 revealed the presence of partial dominance for susceptibly.

Variances and heritability estimate:

Calculated variances (S^2) for parents, F_1 and F_2 of the eight crosses inoculated with *Puccinia triticina* at the adult stage under field conditions were tabulated in (Table 5). The variance values of the susceptible wheat leaf rusting parent thatcher and the eight cultivars used, *i.e.* Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Sids 1, Sids 12, Sids 13 and Sids 14 were 9, 16, 24, 24, 9, 21, 25, 5.64, and 3.84 respectively. The F_1 variance of the eight tested crosses was, 18.24, 21, 16, 21, 9, 21, 21 and 9, respectively (Table 5). The values of the F_2 variances were in general, high for all studied crosses. These values were 257.44, 223.12, 283.07, 28.5, 314.08, 220.7, 263.04 and 64.99 in sequence in addition to the heritability values for all the tested crosses are considered to be higher ranged from 58.11% to 96.11%. (Table 5).

Number of genes:

Leaf rust severity means of parents and variance of F_1 and F_2 were used to quantitative estimate of the number of genes that condition field resistance in the tested wheat cultivars. The results obtained from (Table 5) reveal that the crosses between thatcher and each of cultivars, Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Sids 1, Sids 12, Sids 13 and Sids 14 revealed that the difference between each of all parents are controlled by two or three gene pairs. Since, the calculated numbers of genes were 1.96, 1.25, 0.94, 7.7, 0.10, 2.56, 2.41 and 10.5 respectively. The number of genes to all tested parents are controlled by one or teen genes pairs. In most cases in adult plant stage these rustles indicate that the selection for this character in early segregating generations could be possible. While delaying it would be more effective, due to the important role of dominance effects in the expression of this trait.

Table 5. *Leaf rust severity means, variances, degrees of dominance, heritability in its broad sense (%) and number of genes for 8 bread wheat crosses at adult stage under field conditions in 2018/2019 growing season.*

No.	Cross name	No. of tested Plants	$\overline{X}$	S^2	Degrees of dominance		Heritability (%)	No. of genes
					h1	h2		
1	Thatcher × Gemmeiza 9	P1 50	74.00	9.00	-0.45	-1.9	94.66	1.96
		P2 50	17.00	16.00				
		F_1 50	32.60	18.24				
		F_2 300	17.13	257.44				
2	Thatcher × Gemmeiza 10	P1 50	74.00	9.00	-0.60	-3.14	92.58	1.25
		P2 50	29.00	24.00				
		F_1 50	38.00	21.00				
		F_2 300	45.76	223.12				
3	Thatcher × Gemmeiza 11	P1 50	74.00	9.00				

No.	Cross name	No. of tested Plants	$\overline{X}$	S^2	Degrees of dominance h1	h2	Heritability (%)	No. of genes
		P2 50	29.00	24.00	-0.37	-0.51	94.65	0.94
		F_1 50	43.00	16.00				
		F_2 300	45.76	283.07				
4	Thatcher × Gemmeiza 12	P1 50	74.00	9.00				
		P2 50	6.00	9.00	-0.64	-1.95	58.11	7.06
		F_1 50	18.00	21.00				
		F_2 300	6.70	28.5				
5	Thatcher × Sids 1	P1 50	74.00	9.00				
		P2 50	58.00	21.00	+1	-6.12	96.19	0.10
		F_1 50	74.00	9.00				
		F_2 300	41.50	314.08				
6	Thatcher × Sids 12	P1 50	74.00	9.00				
		P2 50	10.00	25.00	-0.75	-1.54	92.39	2.56
		F_1 50	18.00	21.00				
		F_2 300	17.30	220.70				
7	Thatcher × Sids 13	P1 50	74.00	9.00				
		P2 50	5.60	5.64	-0.63	-1.23	96.11	2.41
		F_1 50	18.00	21.00				
		F_2 300	18.60	263.04				
8	Thatcher× Sids 14	P1 50	74.00	9.00				
		P2 50	5.40	3.84	-0.69	-1.72	89.57	10.5
		F_1 50	16.00	9.00				
		F_2 300	10.06	64.99				

Identification of adult-plant leaf ruts resistance genes in eight wheat cultivars:

Genetic analysis of the six leaf rust monogenic lines and eight bread wheat cultivars was conducted. F_2 plants of the crosses between the tested wheat cultivars and monogenic lines were evaluated at the adult plant stage under field conditions.

Identification of leaf ruts resistance gene; Lr 50 in eight wheat cultivars:

Results of the crosses between the tested wheat cultivars and the monogenic lines at adult plant stage in (Table 6). All of the 432 F_2 plants of the crosses between the adult plant resistance gene *Lr* 50 and the wheat cultivar Gemmeiza 12 were resistant and showed no segregations, indicating that the wheat cultivar Gemmeiza 12 has the leaf rust resistance gene *Lr* 50. The F_2 plants of the crosses between *Lr* 50 and the rest of the tested cultivars, *i.e.* Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Sids 1, Sids 12, Sids 13 and Sids 14 segregated to 305 R: 109 S, 369 R: 124 S, 337 R: 115 S, 339 R: 102 S, 355 R: 111 S, 309 R: 99 S and 384 R: 120 S, respectively. These segregations fit the ratio 3 R: 1 S, indicated that these cultivars do not have *Lr* 50 (Table 6).

Identification of leaf ruts resistance gene; Lr 51 in eight wheat cultivars:

The F_2 plants of the crosses between *Lr* 51 and the eight tested cultivars, *i.e.* Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Sids 1, Sids 12, Sids 13 and Sids 14 segregated to 355 R: 112 S, 379 R: 128 S, 309 R: 100 S, 336 R: 104 S, 355 R: 110 S, 374 R: 129 S, 315 R: 117 S and 395 R: 119 S, respectively. These segregations fit the ratio 3 R: 1 S, indicated that these cultivars do not have *Lr* 51 (Table 6).

Identification of leaf ruts resistance gene; Lr 54 in eight wheat cultivars:

The F_2 plants of the crosses between *Lr* 54 and the eight tested cultivars, *i.e.* Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Sids 1, Sids 12, Sids 13 and Sids 14 segregated to 361 R: 108 S, 407 R: 130 S, 350 R: 122 S, 319 R: 101 S, 377 R: 122 S, 411 R: 139 S, 379 R: 132 S and 355 R: 115 S, respectively. These segregations fit the ratio 3 R: 1 S, indicated that these cultivars do not have *Lr* 54 (Table 6).

Identification of leaf ruts resistance gene; Lr 64 in eight wheat cultivars:

All of the 410 F_2 plants of the crosses between the adult plant resistance gene *Lr* 64 and the wheat cultivar Sids 14 were resistant and showed no segregations, indicating that the wheat cultivar Sids 14 has the leaf rust resistance gene *Lr* 64. The F_2 plants of the crosses between *Lr* 64 and the other tested cultivars, *i.e.* Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Sids 1, Sids 12 and Sids 13 segregated to 381 R: 137 S, 419 R: 131 S, 375 R: 122 S, 349 R: 114 S, 390 R: 128 S, 361 R: 115 S and 388 R: 125 S, respectively. These segregations fit the ratio 3 R: 1 S, indicated that these cultivars do not have *Lr* 64 (Table 6).

Identification of leaf ruts resistance gene; Lr 67 in eight wheat cultivars:

All of the 405 F_2 plants of the crosses between the adult plant resistance gene *Lr* 67 and the wheat cultivar Gemmeiza 12 were resistant and showed no segregations, indicating that the wheat cultivar Gemmeiza 12 has the leaf rust resistance gene *Lr* 67. The F_2 plants of the crosses between *Lr* 64 and the other tested cultivars, *i.e.* Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Sids 1, Sids 12, Sids 13 and Sids 14 segregated to 371 R: 113 S, 420 R: 137 S, 369 R: 124 S, 338 R: 105 S, 363 R: 116 S, 419 R: 130 S and 407 R: 133 S, respectively. These segregations fit the ratio 3 R: 1 S, indicated that these cultivars do not have *Lr* 67 (Table 6).

Identification of leaf ruts resistance gene; Lr 68 in eight wheat cultivars:

The F_2 plants of the crosses between *Lr* 68 and the eight tested cultivars, *i.e.* Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Sids 1, Sids 12, Sids 13 and Sids 14 segregated to 367 R: 118 S, 437 R: 145 S, 420 R: 139 S, 424 R: 140 S, 395 R: 122 S, 370 R: 119 S, 419 R: 137 S and 427 R: 131 S, respectively. These segregations fit the ratio 3 R: 1 S, indicated that these cultivars do not have *Lr* 68 (Table 6).

Table 6. *Segregations and Chi square analysis of F_2 plants of the crosses between six leaf rust monogenic lines and eight bread wheat cultivars at adult plant stage under field conditions at Elbostan location during 2018/19 growing season.*

Cross	No. of F_2 plants		Expected ratio	χ^2	*P.* value
	Resistant (R)	Susceptible (S)			
Gemmeiza 9 × *Lr* 50	305	109	3: 1	0.390	0.532
Gemmeiza 10 × *Lr* 50	369	124	3: 1	0.006	0.938
Gemmeiza 11 × *Lr* 50	337	115	3: 1	0.047	0.828
Gemmeiza 12 × *Lr* 50	432	0	No segregation	-	-
Sids 1 × *Lr* 50	339	102	3: 1	0.823	0.364
Sids 12 × *Lr* 50	355	111	3: 1	0.346	0.556
Sids 13 × *Lr* 50	319	99	3: 1	0.386	0.534
Sids 14 × *Lr* 50	384	120	3: 1	0.381	0.537
Gemmeiza 9 × *Lr* 51	355	112	3: 1	0.258	0.612
Gemmeiza 10 × *Lr* 51	379	128	3: 1	0.016	0.898
Gemmeiza 11 × *Lr* 51	309	100	3: 1	0.066	0.797
Gemmeiza 12 × *Lr* 51	336	104	3: 1	0.436	0.509
Sids 1 × *Lr* 51	355	110	3: 1	0.448	0.503
Sids 12 × *Lr* 51	374	129	3: 1	0.112	0.738
Sids 13 × *Lr* 51	315	117	3: 1	1.000	0.317
Sids 14 × *Lr* 51	395	119	3: 1	0.936	0.333
Gemmeiza 9 × *Lr* 54	361	108	3: 1	0.973	0.324
Gemmeiza 10 × *Lr* 54	407	130	3: 1	0.179	0.672
Gemmeiza 11 × *Lr* 54	350	122	3: 1	0.181	0.671
Gemmeiza 12 × *Lr* 54	319	101	3: 1	0.203	0.652
Sids 1 × *Lr* 54	377	122	3: 1	0.081	0.776
Sids 12 × *Lr* 54	411	139	3: 1	0.022	0.883
Sids 13 × *Lr* 54	379	132	3: 1	0.189	0.664
Sids 14 × *Lr* 54	355	115	3: 1	0.071	0.790
Gemmeiza 9 × *Lr* 64	381	137	3: 1	0.579	0.447
Gemmeiza 10 × *Lr* 64	419	131	3: 1	0.410	0.522
Gemmeiza 11 × *Lr* 64	375	122	3: 1	0.054	0.816
Gemmeiza 12 × *Lr* 64	349	114	3: 1	0.035	0.851
Sids 1 × *Lr* 64	390	128	3: 1	0.023	0.879
Sids 12 × *Lr* 64	361	115	3: 1	0.179	0.672
Sids 13 × *Lr* 64	388	125	3: 1	0.110	0.740
Sids 14 × *Lr* 64	410	0	No segregation	-	-
Gemmeiza 9 × *Lr* 67	371	113	3: 1	0.705	0.401
Gemmeiza 10 × *Lr* 67	420	137	3: 1	0.048	0.826
Gemmeiza 11 × *Lr* 67	369	124	3: 1	0.006	0.938
Gemmeiza 12 × *Lr* 67	405	0	No segregation	-	-
Sids 1 × *Lr* 67	338	105	3: 1	0.398	0.528
Sids 12 × *Lr* 67	363	116	3: 1	0.157	0.692
Sids 13 × *Lr* 67	419	130	3: 1	0.511	0.475
Sids 14 × *Lr* 67	407	133	3: 1	0.040	0.842
Gemmeiza 9 × *Lr* 68	367	118	3: 1	0.116	0.733
Gemmeiza 10 × *Lr* 68	437	145	3: 1	0.002	0.962
Gemmeiza 11 × *Lr* 68	420	139	3: 1	0.005	0.942
Gemmeiza 12 × *Lr* 68	424	140	3: 1	0.009	0.923
Sids 1 × *Lr* 68	395	122	3: 1	0.542	0.462
Sids 12 × *Lr* 68	370	119	3: 1	0.115	0.734
Sids 13 × *Lr* 68	419	137	3: 1	0.038	0.845
Sids 14 × *Lr* 68	427	131	3: 1	0.691	0.406

P. values higher than 0.05 indicate non-significant of χ^2.

4. Discussion

Information on the genetics of resistance to *Puccinia triticina* in wheat can facilitate efficient *Lr* gene exploitation leading to the development of wheat cultivars with high resistance. Combination of several effective resistance genes into a single wheat cultivar can extend the period of resistance since evolution of new virulent races [25].

Inheritance mode of leaf rust resistance at adult plant stage in eight wheat cultivars *i.e.* Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Sids 1, Sids 12, Sids 13 and Sids 14 were studied by crossing among these cultivars and the highly susceptible wheat cultivars Thatcher. However, the two parents, F_1 and F_2 population for each of the eight crosses were tested. The quantitative analysis of the F_1 and F_2 leaf rust severity means in the eight crosses were mostly lower than the estimated means for their respective mid-parents suggested the partial dominance for leaf rust resistance expect for the three crosses *i.e.* Gemmeiza 10, Gemmeiza 11 and Sids 13 which exhibited positive values higher than the respective mid-parents suggested the presence of over dominance toward susceptibility in these crosses. This result was confirmed by those obtained by [7, 8].

High heritability values are indicative for high rates of success in recovering the desired genes in future generations. Also, these high estimated values indicate that the selection for this character in early segregating generations could be possible. While delaying it would be more effective due to the important role of dominance effects in the expression of this trait. These results are in harmony with those of [7, 8, 26, 27].

The number of genes to all tested parents are controlled by two or teen gene pairs. In most cases in adult plant stage these rustles indicate that the selection for this character in early segregating generations could be possible. While delaying it would be more effective, due to the important role of dominance effects in the expression of this trait. These results agree with [8, 28, 29].

To identify the gene (s) governing resistance to leaf rust in a wheat cultivar; there are three methods *i.e.* gene postulation, genetic analysis and using molecular markers. Although it is possible to hypothesize which *Lr* resistance genes may be present in a wheat cultivar based on gene postulation method, the specific number and identity of resistance genes in wheat cultivars only be determined conclusively by genetic analysis [30]. Basically, genetic studies and analysis of breeding generations such as F_2 populations are conducted to identify the resistance gene (s) present in different cultivars.

This experiment included crossing of the tested eight wheat cultivars with six leaf rust monogenic lines*, i.e. Lr* 50, *Lr* 51, *Lr* 54, *Lr* 64, *Lr* 67 and *Lr* 68. The leaf rust resistance gene *Lr* 50 was introgressed from *T. timopheevii* sub sp. *armeniacum* and mapped on the chromosome 2BL with flanking SSR markers *Xgwm382* and *Xgdm87* [31]. Virulence to *Lr* 50 exists in races of *P. triticina* in North America. The leaf rust races; PNMQ and MBRL were virulent on seedlings of lines having *Lr* 50. However, low to intermediate infections types have been observed on adult plants of wheat having this gene when evaluated for multiple years under field conditions indicating that virulence is not common in the Southern U.S. Great Plains. Selection pressure on the pathogen imposed by deployment of genes such as *Lr* 9, *Lr* 24, and *Lr 41* that were also ineffective against the PNMQ race of *P. triticina* may result in an increase in virulence to *Lr* 50 in the region. This will limit the usefulness of this gene unless it is deployed in combination with other effective genes [32].

The leaf rust resistance gene; *Lr* 51 was transferred from *Triticum speltoides* to common wheat chromosome and located on arm 1BL, this gene, temporarily named *Lr* F7, has been designated *Lr* 51 [33]. Although, it has high levels of resistance to predominant leaf rust races, not been widely deployed in breeding programs, probably because of negative genetic effects associated with the presence of large *T. speltoides* chromosome segments and/or additional homeologous translocations in other wheat chromosomes. Plants homozygous for *Lr* 51 were highly resistant with hypersensitive flecks, whereas heterozygous plants showed slightly lower levels of resistance with small pustules surrounded by necrosis and chlorosis, indicating incomplete dominance [34].

The leaf rust resistance gene; *Lr* 54 was translocated to chromosome 2DL of common wheat. There is linked between this gene and stripe rust resistance gene *Yr* 37, respectively. The leaf rust resistance gene *Lr* 64 located on chromosome arm 6AL.

The leaf rust resistance gene; *Lr* 67 is located on chromosome 4DL with a tightly linkage between it and each of stripe rust resistance gene *Yr* 46, stem rust resistance gene *Sr* 55, powdery mildew resistance gene *Pm* 46 and leaf tip necrosis gene *Ltn* 3 [35].

Finally, *Lr* 68 is located on chromosome 7BL and also linked with leaf tip necrosis gene [36]. The previous reports showed in general, that pyramiding of *Lr* 34, *Lr* 46, *Lr* 67 and *Lr* 68 in different combinations within a particular wheat genotype confers high and/or sustainable level of resistance to wheat leaf rust and also expected to be long-lasting or more durable [37].

Genetic analyses of the crosses of the wheat cultivars; Gemmeiza 10, Sids 13 and Sids 14 with the wheat variety; Thatcher showed the ratio 3 R: 1 S. Moreover, these cultivars showed 1.25, 1.41 and 1.5 genes, respectively, while they crossed with the indicating that these cultivars had one dominant gene. Results of this study showed that, the wheat cultivar Sids 14 has *Lr* 64. Moreover [12] reported that these wheat cultivars; Gemmeiza 10 and Sids 13 have only one gene *i.e. Lr* 46 and *Lr* 34, respectively. On the other hand, the genetic analyses of the wheat cultivars; Gemmeiza 9, Gemmeiza 12 and Sids 12 showed the ratio 15 R: 1 S. Moreover, indicating that these cultivars have two dominant genes. Data of this study showed that the wheat cultivar; Gemmeiza 12 had *Lr* 50. Also [12] found that these wheat cultivars; Gemmeiza 9, Gemmeiza 12 and Sids 13 had one gene *i.e. Lr* 46, *Lr* 67 and *Lr* 46, respectively. Moreover, the wheat cultivars; Gemmeiza 9 and Sids 13 may have another untested gene.

Genetic analyses of the crosses of the wheat cultivars; Sids 1 and Gemmeiza 11 showed the ratio 1 R: 15 S and 1 R: 63 S, respectively. These indicated that these cultivars had two recessive genes and three recessive genes, respectively and same results were reported by [38-43].

Our further study will be using molecular markers to detect the above six leaf rust resistance genes in these wheat cultivars. Available markers should be verified with combined studies of field resistance to rust fungi. New biotechnological methods complement the conventional wheat breeding program and these new methods enrich conventional breeding methods but cannot replace them [44-46].

5. Conclusion

Using resistance genes to leaf rust caused by *Puccinia triticina* is the most economical, effective practice, and sustainable disease management strategy. Results showed that the wheat cultivar Gemmeiza 12 has the two leaf rust resistance genes; *Lr* 50 and *Lr* 67 and the wheat cultivar Sids 14 has resistance gene; *Lr* 64. While, the other tested wheat cultivars doesn't carry any of the tested leaf rust resistance genes at adult-plant stage. These resistance genes can be used in the breeding program to improve wheat resistance to leaf rust disease.

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