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ORIGINAL PAPER

Detection and Introgression of *Lr*46 Gene-Conferring Partial Resistance to Leaf Rust (*Puccinia triticina*) in Wheat

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ABSTRACT

Detection and introgression of slow leaf rust resistance gene, *Lr*46 in seven parental wheat cultivars, *i.e.*, Gemmeiza-9, Gemmeiza-11, Gemmeiza-12, Misr-1, Misr-2, Sakha-94 and Giza-171 and their F_1 and F_2 crosses was carried out at Bahteem Agricultural Research Station, Qaliobia governorate, Egypt. These tested cultivars varied in their reactions to leaf rust disease. The cultivars Misr-1, Misr-2, Sakha-94, and Giza-171 showed slow rust reaction meanwhile, Gemmeiza-9, Gemmeiza-11 and Gemmeiza-12 cultivars were fast rusting. Values of area under disease progress curve (AUDPC) run in a parallel line with its disease severity. *Lr*46 gene was found in cultivars Misr-1, Misr-2, Sakha-94, and Giza-171 and absent in cultivars Gemmeiza-9, Gemmeiza-11, Gemmeiza-12 cultivars at 300 pb. Qualitative analysis of the obtained data showed that there were no segregations in the crosses *Lr*46×Misr-1, *Lr*46×Misr-2, *Lr*46×Sakha94 and *Lr*46×Giza-171 in the F_2 plants, indicating the presence of the *Lr*46 in these cultivars. While, segregation was found in the crosses *Lr*46×Gemmeiza-9, *Lr*46×Gemmeiza-11, *Lr*46×Gemmeiza-12, indicating that these cultivars do not carry *Lr*46 gene. Additionally, the heritability was above 94%, suggesting that the selection of genotypes resistant to leaf rust in the first generations (F_2) was feasible. However, this selection is more successful in later generations because the dominance effect is critical for the expression of this trait. Molecular detection proved that *Lr*46 gene was inserted into Gemmeiza-9, Gemmeiza-11 and Gemmeiza-12 to have slow rust resistance in F_2 plants. This saves effort and time as it is possible to rely on molecular marker assisted selection in the early selection of plants carrying this gene. Mean of the yield components, plant height, number of spikes/plant, number of kernels/plant, 100-kernel weight, and grain yield/plant of the three F_2 crosses was higher than those of their parents. So, plant breeders should not rely on complete rust resistance, but should consider partial resistance, particularly slow rusting resistance genes.

Keywords: Wheat, *Triticum aestivum*, leaf rust, *Puccinia triticina*, *Lr*46 Gene, slow rusting, partial resistance.

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INTRODUCTION:

In Egypt, wheat (*Triticum aestivum* L.) is the most significant crop grown for food consumption. It is grown in approximately 3.4 million feddans annually, yielding about 9.8 million tons of wheat grains (Wally and Seifarth 2022). Wheat is exposed annually to some fungal diseases, especially the rust diseases (yellow, leaf, and stem rusts). One of the most significant wheat diseases in Egypt and worldwide is leaf rust (*Puccinia triticina* Eriks), which is adaptable to a wide range of environments where wheat is grown (Roelfs *et al*., 1992). The grain yield loss can reach approximately 23% or more in the susceptible cultivars based on the crop development stage of rust infection, resulting in a significant reduction in grain yield (Ali *et al*., 2016). According to Pink (2002), genetic resistance is the most practical and cost-effective way to lessen the production loss brought on by the leaf rust. High yielding and resistance bread wheat cultivars were introduced by incorporation between both the Wheat Research Department, Crops Research Institute, and the Department of Wheat Diseases Research Department, Plant Pathology Research Institute, ARC, Egypt *i.e*., Giza-171 (Hamada *et al*., 2015), Sids-14 (Abd El-Majeed *et al*., 2017), Misr-3 (Abd El-Majeed *et al*., 2018), and Misr-4 (Kumber *et al*., 2022).

Wheat breeders have generally relied on leaf rust genes for hypersensitivity tolerance (HR) to reduce the accumulation of epidemics and is easy to administer in breeding programmes. As a result, due to appearance of new virulent races that may breakdown the resistance of wheat cultivars, it must be knowing the resistance genes in parental germplasm to improve the effectiveness of developing new resistant germplasm (Omara *et al*., 2021). The number of leaf rust resistance genes (Lrs) has increased to

over 74, and the majority of them are mapped on various chromosomes (McIntosh *et al*., 2013). The ability to effectively incorporate various genes into germplasm pools is made possible by the identification of the leaf rust resistance genes, preventing the introduction of cultivars with identical genetic makeup (Kolmer, 1996).

There are two types of plant disease resistance: quantitative resistance, which is mediated by a number of genes or quantitative trait loci (QTLs), each of which increases a partial resistance, and qualitative resistance, which is mediated by a single resistance gene (Kaur and Bariana, 2010). Persistent resistance is primarily caused by quantitative resistance, which postpones the growth and emergence of wheat leaf rust. Typically, qualitative resistance is less tenacious than quantitative resistance. Rust resistance genes have only a low to moderate effect when present alone, but by combining four to five genes, substantial levels of resistance have been attained (Singh *et al*., 2000). The strategy of pathologists and wheat breeders is to increase wheat yield by genetically improving wheat cultivars and the level of adult plant resistance against rusts (El-Orabey *et al*., 2020). Geneticists and plant breeders have therefore stressed the significance of creating and using late-deploying cultivars that have long-lasting or slow rust resistance by quantitatively inheriting several genes (Parlevliet 1975, Kaur and Bariana 2010, and Omara *et al*., 2021).

When combined with the *Yr*29 gene, the leaf rust resistance gene *Lr*46 has also produced long-lasting resistance to stripe rust and leaf rust (Singh *et al*., 2005). Singh *et al*. (1998) mentioned that *Lr*46 is the gene symbol that is localised on chromosome 1B in the Pavon-76 cultivar, which has remained effective since its release. Partial resistance (PR) is important in the wheat breeding program, which is dependent on the slow spread of epidemics of highly infectious types (Parlevliet, 1975 and Omara *et al*., 2017). The genetic pyramid of leaf rust genes is necessary to obtain the long-term durability of leaf rust resistance in Egypt (Atia *et al*., 2021). Therefore, the current study aims to detect and introgress the slow rust resistance gene, *Lr*46, in seven Egyptian wheat cultivars by genetic analysis and molecular markers, as well as improve yield parameters in wheat hybrids.

MATERIALS AND METHODS

Plant Materials:

Seven bread wheat cultivars, Gemmeiza-9, Gemmeiza-11, Gemmeiza-12, Misr-1, Misr-2, Sakha-94 and Giza-171, as well as *Lr*46 (Pavon 76) were tested in the field at Bahteem Agricultural Research Station, ARC, Qaliobia governorate, Egypt during 2019/2020 and 2020/2021 seasons. Each genotype was sown in plots consists of four rows, 3m long and 30cm apart and planted with 5g of seed per row. A random complete block design with three replicates for each was used in the experiment. Morocco variety served as a spreader of leaf rust, which was inoculated by a mixture of PPKST, PSTDT, and TTTSK physiological races during the late elongation and late tillering stages.

Disease assessment:

Disease severity (DS) was measured four times, every 10 days, according to Peterson *et al*., (1948), as the percentage of leaves covered with rust pustules. According to Stakman *et al*. (1962), the infection types were recorded as immune = (0), resistant = (R), moderately resistant = (MR), moderately susceptible = (MS), and susceptible = (S). The above data were used to calculate the final rust severity (FRS) and area under the disease progression curve (AUDPC) (Pandey *et al*., 1989).

Qualitative and Quantitative analysis:

Seven crosses were conducted between *Lr*46 gene (male parent) and the previous bread wheat cultivars (female parents) at Bahteem Agricultural Research Station, Qaliobia governorate to obtain hybrid seeds in 2019/2020 season. In 2020/2021 season, seeds of the seven F_1 hybrids crosses were sown in one row, 2m long, 30 cm apart and 10 cm within rows with 4 randomized complete block design to have F_1 seeds per each cross.

Assessment F_1 and F_2 plants in 2021/2022 season:

At seedling stage:

Seeds of each hybrid and F_1 cross were planted in 10 pots (25 cm diameter), 25 seed/ pot in the leaf rust greenhouse of the Wheat Diseases Research Department, ARC, Giza. According to Tervet and Cassell (1951), the materials were inoculated by the urediniospores of the TTTSK race 7 days after planting by dusting a mixture of the spores and talcum powder in a ratio of 1:20 (v/v). On the first leaves, 12 days after planting, the infection type of rust reaction was recorded using the scale of Stakman *et al*. (1962), where R = (0, 1, 2) and S = (3, 4).

At adult stage:

Seeds of each hybrid and F_1 crosses were planted in plots consisted of 12 rows for each (3m length and 30 cm apart). The plots were bordered by border rows of the highly sensitive variety Morocco, dusted by a mixture of TTTKS spores

and powder talcum (1:20) based on the approach by Tervet and Cassel (1951), at the late tellering and late elongation stages (middle of February). Disease severity % on F_1 and F_2 plants of each cross was recorded onset appearing leaf rust on any plant. The F_2 plants were divided into six classes depending on disease severity according to Peterson *et al*., (1948) and the infection types (Roelfs *et al*., 1992). Plants with (0–10, 11–20, 21–30) disease severity were considered to have low phenotypes, while plants with (31–40, 41-50 and 51–60) severity % were seen to have high phenotypes. Chi-square tests were conducted to test the goodness of fit of the F_2 population between observed and expected segregation ratios by the formula of Steel and Torrie, (1960) as follows:

$$\chi c^2 = \Sigma\ (Oi - Ei)2/Ei$$

Where:

C = Degrees of freedom,

Oi = Observed value(s),

Ei = Expected value(s)

Frequency distribution of disease severity

Frequency distribution values were calculated for the severity of leaf rust under field conditions for the parents, F_1 and F_2 populations. According to Steel and Torrie (1960), chi-square (X^2) analysis was used to establish the mode of inheritance's goodness of fit between the observed and anticipated proportions of the phenotypic class (% leaf rust severity). Wright (1968) also claimed that the minimal set of genes required to govern resistance were determined.

Romero and Frey (1973) approach was used to determine the degrees of dominance. The following formula was used in this procedure to determine the degree of dominance denoted as h_1 and h_2 for F_1 and F_2, respectively:

$$\mathbf{h_1} = (\overline{\mathbf{X}}\mathbf{F_1} - \overline{\mathbf{X}}\,\mathbf{MP})/\mathbf{D} \text{ and } \mathbf{h_2} = \mathbf{2}(\overline{\mathbf{X}}\mathbf{F_2} - \overline{\mathbf{X}}\,\mathbf{MP})/\mathbf{D}$$

Where:

$$\mathbf{D} = (\overline{\mathbf{X}}\,\mathbf{hp} - \overline{\mathbf{X}}\,\mathbf{MP})$$

$\overline{X}$ F_1, $\overline{X}$ F_2 and $\overline{X}$ h_p are the means of F_1, F_2 and high parent, sequently, while the mid-parent value is $\overline{X}$ MP.

To ascertain whether the h_1 and h_2 values were significantly different from zero, the F_1 and F_2 averages were also compared with the mid-parent value using the t test. Lush (1949) formula was used to evaluate heritability in the broad sense.

Molecular detection of *Lr*46 in the bread wheat cultivars and F_2 crosses.

Molecular detection of *Lr*46 gene in the previous seven wheat cultivars and the resistant F_2 plants were performed. Genomic DNA was extracted from young leaves of two weeks old plants using the methods of Dellaporta *et al*. (1983). Thereafter, DNA concentration was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), the purity and concentration of the DNA were assessed. The DNA concentration was then increased and adjusted to 50 ng µL-1 for additional PCR amplification. The specific primer (F: 5` - GGT CTT CTG GGC TTT GAT CCT-3` and R: 5`-GTT GCT AGG GAC CCG TAG TGG -3`), created for *Lr*46-associated markers, was used in the PCR amplification in a 25 µL reaction mixture (Paillard *et al*., 2003). 2.5 µL of genomic DNA (50 µL−1), 1.0 µL of each forward and reverse primers (10 picomoles), and 8.0 µL of Milli-Q-H_2O made up the PCR reaction mixture. After optimization, a thermal cycler was used to carry out DNA amplification (Rocorbett-Research, CG1-96). The thermocycling condition for PCR starting with one cycle of denaturation at 95 °C for 5 min, followed by 35 cycles each of denaturation at 95 °C for 30 sec., annealing at 64 °C for 30 sec., and extension at 72 °C for 30 sec. Additionally, a last extension step lasting 7 minutes at 72°C was completed and then held at 4°C. Amplified DNA products were run on 1.2%(w/v) agarose gel stained with ethidium bromide (500 µL-1) and separated by electrophoresis at 100 V/1 h, PCR products were resolved. The molecular weight of the investigated materials was determined using a mid-range DNA ladder 100 bp - 3 kbp linear assay (Jena Bioscience, Jena, Germany).

Plant height and yield components:

Plant height and yield components, such as, number of spikes per plant, number of kernels per plant, 100-kernel weight (g), and grain yield per plant (g) of the three crosses; *Lr*46×Gemmeiza-9, *Lr*46×Gemmeiza-11 and *Lr*46×Gemmeiza-12 were assessed in F_2 populations. The significance of parent differences was examined using the t-test. Data from the parents and their F_1 and F_2 plants were used to calculate the phenotypic, genotypic, and environmental variations (Acquaah, 2012). The F ratio was used to determine whether the differences between the F_2 variance and the parallel environmental variance were significant.

RESULTS

Leaf rust disease development on wheat cultivars and *Lr*46 in the adult stage:

Disease severity for leaf rust was recorded on all studied wheat cultivars (Gemmeiza-9, Gemmeiza-11, Gemmeiza-12, Misr-1, Misr-2,

Sakha-94 and Giza-171) and *Lr*46, after artificial inoculation. There were variations in the percentage of disease severity among the cultivars under study. Gemmeiza-9 and Gemmeiza-11 cultivars developed disease more quickly than Gemmeiza-12, where the highest AUDPC values ranged from 415 to 1200 during 2019/2020 and 2020/2021 growing seasons. While, the development of leaf rust was slow with Misr-1, Misr-2, Sakha-94, Giza-171 and *Lr*46, where AUDPC values ranged from 116 to 265 of the two seasons (Fig. 1). Additionally, leaf rust disease development was faster in the second season.

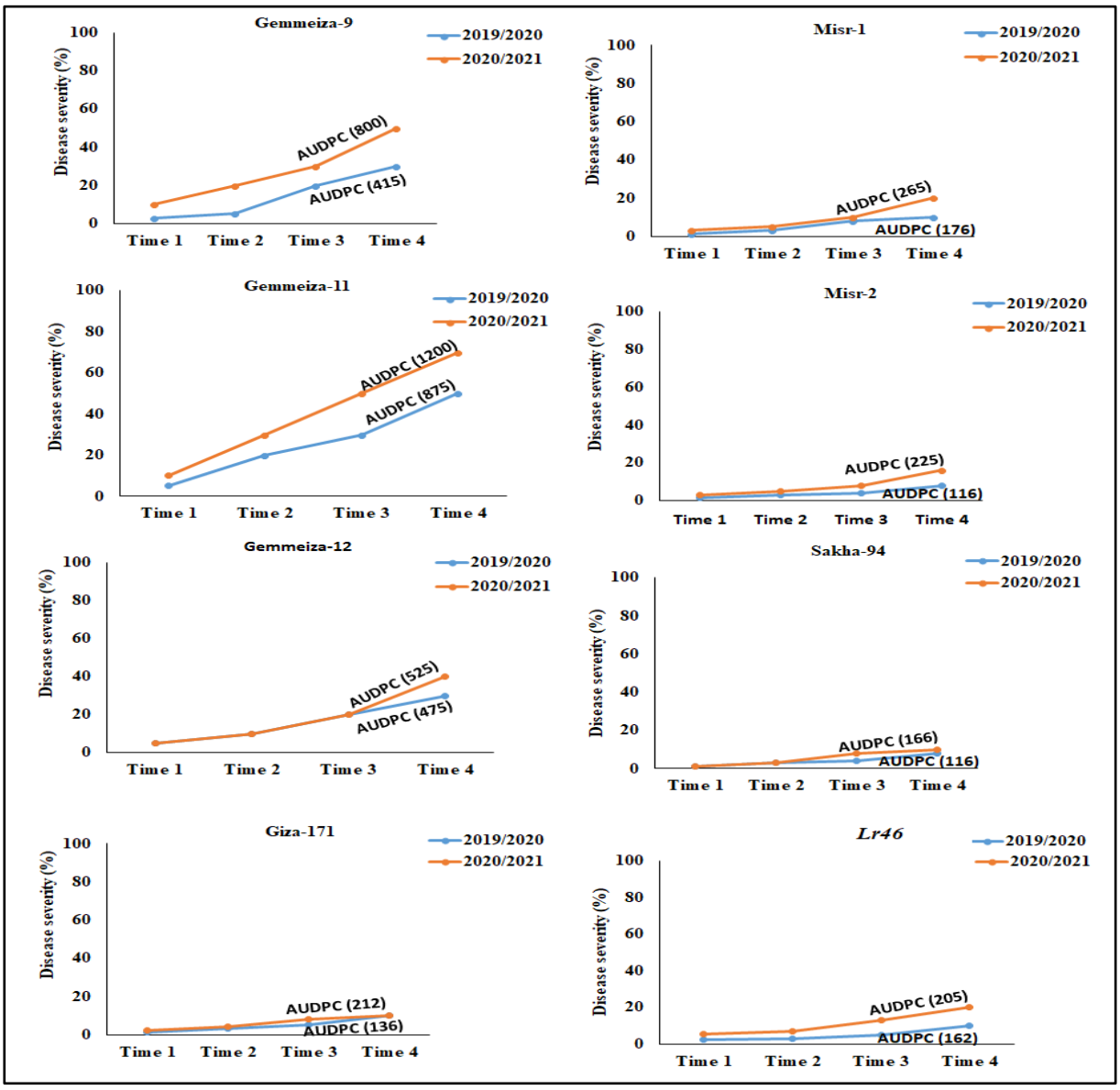

Fig. (1). Disease severity development and area under disease progress curve (AUDPC) of leaf rust on seven wheat cultivars and *Lr*46 during 2019/2020 and 2020/2021 growing seasons.

Qualitative analysis:

Seven crosses between the seven wheat cultivars and *Lr*46 were carried out in order to identify and introgression the slow rusting resistance gene, *Lr*46 at seedling and adult stages (Tables 1, 2 and Figs. 2, 3). At seedling stage, by using chi-square analysis for F_2 plants, the observed and expected ratios of the phenotypic classes for the severity of leaf rust (%) were determined. The obtained results demonstrated that there was no segregation in the F_2 plants of the cross between the three cultivars (Misr-1, Misr-2, Giza-171) and *Lr*46 (Table 2 and Figs. 2, 3). These findings demonstrated the existence of *Lr*46 in Misr-1, Misr-2, and Giza-171 cultivars. As opposed to this, F_2 cross between *Lr46* and Gemmeiza-9, Gemmeiza-11, Gemmeiza-12, and Sakha-94 was segregated into the ratios: 50R: 153S, 11R: 196S, 153R: 47H, and 196R: 18S, respectively. These ratios were 1:3, 1:15, 3:1, and 15:1, respectively (Table 1).

For the seven crosses under study at the adult stage, the frequency distribution of the disease severity (%) in F_2 plants ranged from 0 to 60%. F_2 wheat plants of crosses, *Lr*46×Misr-1, *Lr*46×Misr-2, *Lr*46×Sakha-94 and *Lr*46×Giza-171 showed a low disease severity of leaf rust (L) and showed no segregation. On the other hand, F_2 plants of crosses, *Lr*46×Gemmeiza-9, *Lr*46×Gemmeiza-11 and *Lr*46×Gemmeiza-12 were segregated into 53L: 174H, 47L: 154H, and 176L: 69H, with P_b values of 0.565, 0.597 and 0. 0.253, respectively. These observed ratios fit the expected ratios: 1:3, 1:3, and 3:1 for the above-mentioned seven crosses, respectively (Table 2).

Table (1): Leaf rust frequency distribution of parents, seven F_1 and F_2 crosses, evaluated under artificial inoculation with *P. triticina*, at seedling stage during 2021/2022 growing season.

Cross name		No. of tested plants	Infection type						observed ratio		Expected ratio	X^2	P_b
			0	0;	1	2	3	4	R	S			
*Lr*46×Gemm.-9	P_1	31				31							
	P_2	34						34					
	F_1	35				15	20						
	F_2	203	0	0	23	27	80	73	50	153	1:3	0.015	0.903
*Lr*46×Gemm.-11	P_1	37				37							
	P_2	28						28					
	F_1	34				4	30						
	F_2	207	0	0	0	11	93	103	11	196	1:15	0.310	0.578
*Lr*46×Gemm.-12	P_1	38				38							
	P_2	32					32						
	F_1	35				20	15						
	F_2	200	20	33	50	50	27	20	153	47	3:1	0.240	0.624
*Lr*46×Misr-1	P_1	29				29							
	P_2	42	42										
	F_1	38		24	14								
	F_2	210	88	83	19	20			210	0	No segregation		
*Lr*46×Misr-2	P_1	31				31							
	P_2	35					35						
	F_1	32	18	14									
	F_2	201	76	75	44	6			201	0	No segregation		
*Lr*46×Sakha-94	P_1	30				30							
	P_2	28				28							
	F_1	35		10	25								
	F_2	214	32	62	82	20	9	9	196	18	15:1	1.706	0.192
*Lr*46×Giza-171	P_1	33				33							
	P_2	39					39						
	F_1	30	20	10									
	F_2	208	157	41	10				208	0	No segregation		

R = Resistant (0, 0, 1, 2) and S = Susceptible (3, 4)

Table (2). Leaf rust frequency distribution of parents and seven F_1 and F_2 crosses, evaluated under artificial inoculation with *P. triticina*, at adult stage during 2021/2022 growing season.

Cross name		No. of tested plants	Rust severity (%) classes								Expected ratio	X^2	P_b
			Resistant (R)				Susceptible (S)		observed ratio				
			0-10	11-20	21-30	31-40	41-50	51-60%	L	H			
*Lr*46×Gemm.-9	P_1	32	30	2									
	P_2	43					3	40					
	F_1	30			5	25							
	F_2	227	9	23	21	56	54	64	53	174	1:3	0.330	0.565
*Lr*46×Gemm.-11	P_1	29		25	4								
	P_2	34					30	4					
	F_1	35			13	22							
	F_2	201	11	17	19	44	70	40	47	154	1:3	0.280	0.597
*Lr*46×Gemm.-12	P_1	36	4	32									
	P_2	33			3	30							
	F_1	32	20	12									
	F_2	245	120	50	6	50	10	9	176	69	3:1	1.307	0.253
*Lr*46×Misr-1	P_1	37	7	30									
	P_2	34	30	4									
	F_1	38	26	12									
	F_2	216	130	50	36				216	0	No segregation		
*Lr*46×Misr-2	P_1	38	3	35									
	P_2	36	32	4									
	F_1	34	22	12									
	F_2	212	180	20	12				212	0	No segregation		
*Lr*46×Sakha -94	P_1	32	2	30									
	P_2	29	25	4									
	F_1	30	25	5									
	F_2	220	180	35	5				220	0	No segregation		
*Lr*46×Giza-171	P_1	41	3	38									
	P_2	39	4	35									
	F_1	36	22	14									
	F_2	232	180	30	22				232	0	No segregation		

L = Low rust severity ≤ 30 %
H = High rust severity ≥ 30%

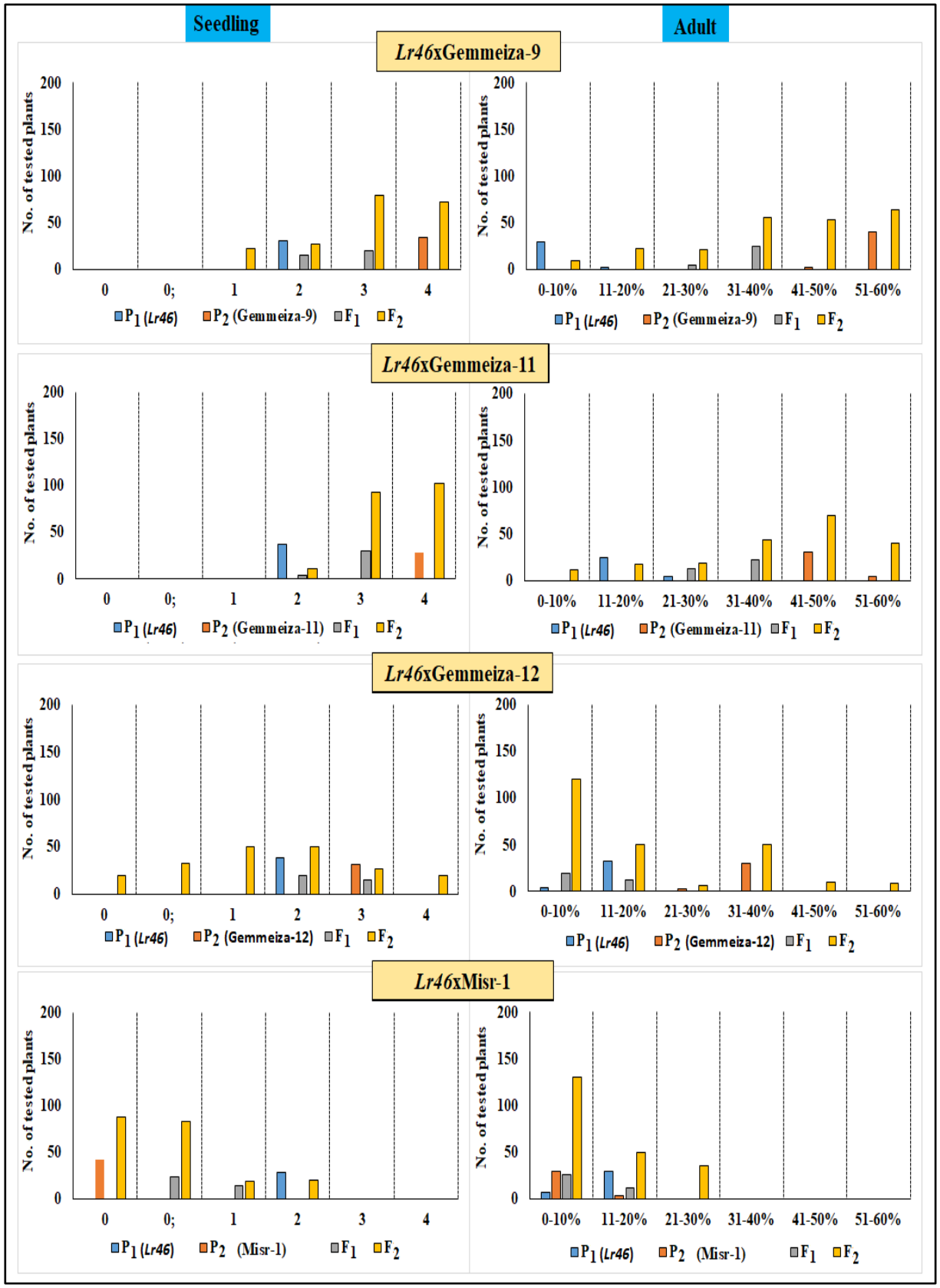

Fig. (2): The frequency distribution of leaf rust severity (%) of four wheat crosses (P_1, P_2, F_1 and F_2) between *Lr*46 and each of Gemmeiza-9, Gemmeiza-11, Gemmeiza-12 and Misr-1 inoculated with *P. triticina* at seedling and adult stages.

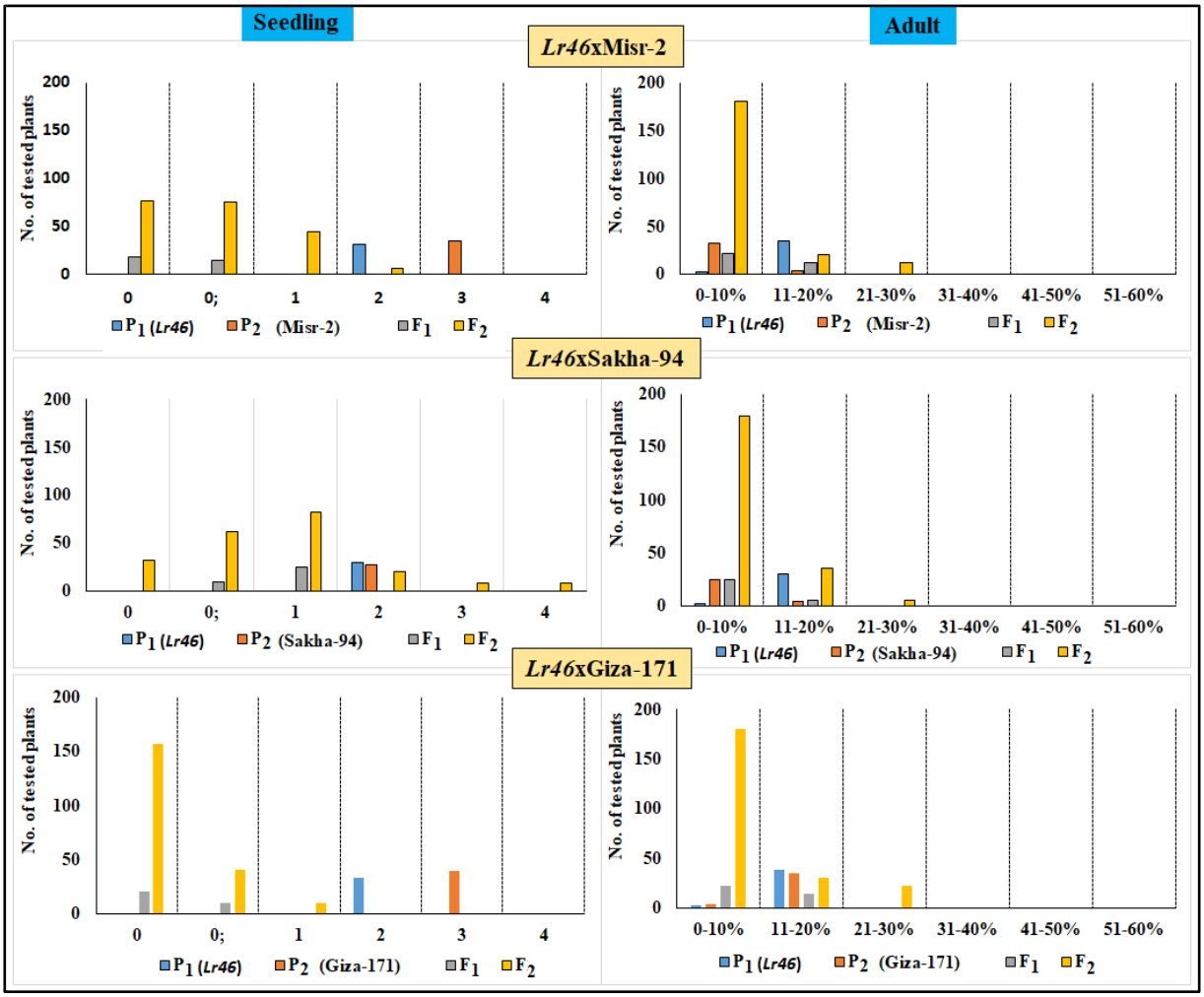

Fig. (3): The frequency distribution of leaf rust severity (%) of three wheat crosses (P_1, P_2, F_1 and F_2) between *Lr*46 and each of Misr-2, Sakha-94 and Giza-171 inoculated with *P. triticina* at seedling and adult stages.

Quantitative analysis:

The two parents, F_1 and F_2 populations, for each of the three crosses (*Lr*46×Gemmeiza-9, *Lr*46×Gemmeiza-11 and *Lr*46×Gemmeiza-12) were tested in the field at the adult plant stage, to ascertain the genetic characteristics of leaf rust resistance quantitatively (Table 3). To determine the level of dominance for the F_1 (h_1) and F_2 (h_2), the means and variances of the parents' respective F_1 and F_2 generations were employed. Additionally, the number of functional resistance genes for each cross and the proportion of heritability in broad-sense (h_2) were estimated (Table 3).

Means and degrees of dominance: the average of disease severity in the crosses *Lr*46×Gemmeiza-9, *Lr*46×Gemmeiza-11 and *Lr*46×Gemmeiza-12 ranged from 13.88 to 54.30% (Table 3). Mean of disease severity of F_1 plants in the three crosses was 15.62, 16.37 and 8.75%, respectively. Furthermore, the disease severity means of F_2 in the three crosses were 30.87, 32.21, and 12.12%, respectively.

Expression of gene effects is measured as h_1 and h_2 as degrees of dominance. The values of h_1 were -0.084, +0.006, and -2.00 in three consecutive crosses, respectively. Significantly negative values for h_1 indicated the existence of partial resistance domains. While, the values of the dominant degree of F_1 (h_1) were +0.006 in the hybrid *Lr*46×Gemmeiza-11. The values of dominance degree F_2 (h_2) were +0.404, +0.130, and -0.729, respectively (Table 3).

Variances and heritability estimate: the variance values of parents ranged from 5.85 to 11.89 for Gemmeiza-9, Gemmeiza-11, Gemmeiza-12 and *Lr*46 (Table 3). The F_1 variances of the three crosses tested were 13.88, 23.34, and 23.43, respectively. F_2 variance values were generally high for all crosses tested. These values were 206.99, 258.90, 224.06 for the three crosses. Moreover, the heritability (%) was high, above 94% for the three crosses. The predicted resistance gene counts for these crosses were, 0.968, 0.440, and 0.064, respectively (Table 3).

PCR based Molecular markers:

Molecular markers were utilized to identify *Lr*46 in seven wheat cultivars, *i.e.*, Gemmeiza-9, Gemmeiza-11, Gemmeiza-12, Misr-1, Misr-2, Sakha-94 and Giza-171 (Fig. 4A). *Lr*46 was only found in Misr-1, Misr-2, Giza-171, and Sakha-94 cultivars and was not present in Gemmeiza-9, Gemmeiza-11, and Gemmeiza-12 (Fig. 4A). While the introgression of *Lr*46 was confirmed in the F_2 plants of the crosses *Lr*46×Gemmeiza-9, *Lr*46×Gemmeiza-11 and *Lr*46×Gemmeiza-12 at 300pb. (Fig.4B).

Table (3): Leaf rust severity means, variances, degrees of dominance, heritability in broad sense (%) and number of genes for three crosses at adult plant stage, during 2021/22 growing season.

Cross name	No. of tested Plants	$\bar{X}$	S^2	Degrees of dominance		Heritability %	No. of genes
				h_1	h_2		
*Lr*46×Gemm.-9	P_1	54.30	6.48				
	P_2	33.33	5.85				
	F_1	15.62	13.88	-0.084			
	F_2	30.87	206.99		+0.404	96.09	0.968
*Lr*46×Gemm.-11	P_1	46.17	10.38				
	P_2	31.28	11.89				
	F_1	16.37	23.34	+0.006			
	F_2	32.21	258.90		+0.130	94.50	0.440
*Lr*46×Gemm.-12	P_1	24.09	8.26				
	P_2	13.88	9.87				
	F_1	8.75	23.43	-2.00			
	F_2	12.12	224.06		-0.729	94.47	0.064

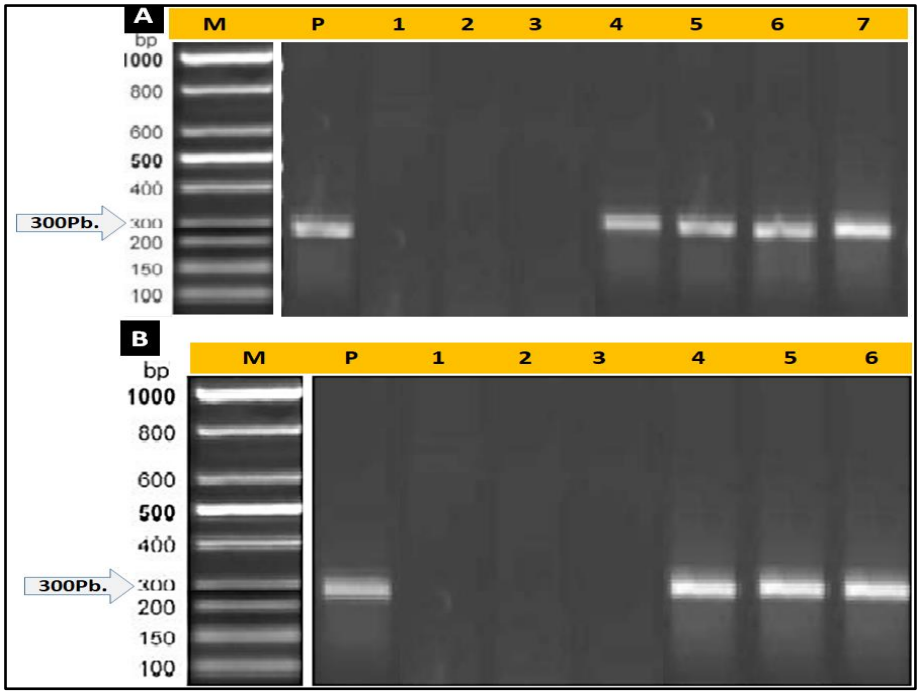

Fig.4 (A and B): Electropherogram profile exhibiting the presence of *Lr*46 marker in the amplified DNA extracted from the 7 cultivars. (A): M= DNA Ladder (DNA Marker), P= Positive, Lane 1= Gemmeiza-9, Lane 2= Gemmeiza-11, Lane 3= Gemmeiz-12, Lane 4= Misr-1, Lane 5= Misr-2, Lane 6= Sakha-94 and Lane 7= Giza-171. (B): P= Positive, Lane 1= Gemmeiza-9, Lane 2= Gemmeiza-11, Lane 3= Gemmeiz-12, Lane 4= *Lr*46×Gemmeiza-9, Lane 5= *Lr*46×Gemmeiza-11 and Lane 6= *Lr*46×Gemmeiza-12.

Plant heigh and yield components:

Due to the importance of the yield parameters**,** the crop characteristics of the three crosses, *Lr*46×Gemmeiza-9, *Lr*46×Gemmeiza-11 and *Lr*46×Gemmeiza-12 were studied. Data in Table (4) show the descriptive statistics of the studied characters for the three crosses of the parents, F_1 and F_2 populations. The parents differed significantly ($P_{0.05}$) for all characters and revealed different genetic backgrounds. The mean values of plant height, number of spikes per plant, number of kernels per plant, 100-kernel weight (g), and grain yield per plant (g), for F_1 values were greater than or nearby to the equivalent to the high parent's values of the three crosses. For yield parameters, the mean of F_2 was higher than the means of the three parents (Table 4).

Tabulated date (Table 5) display several genetic characteristics for the three crosses. For every feature, the phenotypic variations in the F_2 generation of the three crosses were significantly different from the matching environmental variances. Additionally, plant height, spike number, kernel number, and grain yield/plant showed the highest phenotypic, genotypic, and environmental variations. For every character under study, the genetic variance was greater than the matching environmental variance. Additionally, the heritability values for all characters ranged from moderate to high. Modified pedigree/bulk and selected bulk are suggested techniques in the three crosses due to the significant role of genetic variances compared to phenotypic ones and the moderate to high broad-sense heritability's for the majority of studied characters, which indicate the effectiveness of selection in the early generations.

Table (4): Characteristic descriptions for *Lr*46, Gemmeiza-9, Gemmeiza-11 and Gemmeiza-12, as well as their F_1 and F_2 populations in 2021/2022 growing season.

Parent/ generation		Plant height (cm)	No. of spikes/ plant	No. of Kernels/ spike	100-kernel weight (g)	Grain yield/ plant (g)
*Lr*46 (P_1)	Mean	85.40	8.30	65.20	2.20	19.2
	SE	0.41	0.20	1.10	0.06	1.02
	Variance	17.20	3.23	56.10	0.31	59.34
Gemm.-9 (P_2)	Mean	100.13	13.10	65.30	4.56	43.20
	SE	0.21	0.42	0.32	0.04	1.21
	Variance	9.50	25.12	14.34	0.16	152.32
F_1	Mean	101.20	14.20	75.30	3.02	34.60
	SE	0.40	0.33	2.14	0.03	1.31
	Variance	20.05	15.23	225.40	0.02	161.21
F_2	Mean	98.12	10.10	84.60	4.74	34.20
	SE	1.02	0.35	1.11	0.04	1.03
	Min	48.00	5.00	27.00	2.21	13.00
	Max	128.00	23.00	102.4	4.88	88.00
Gemm.-11 (P_3)	Mean	109.67	16.20	76.60	6.23	52.40
	SE	0.42	0.31	0.33	0.04	1.44
	Variance	13.10	30.24	18.54	0.16	177.32
F_1	Mean	118.30	16.30	92.30	4.02	41.30
	SE	0.30	0.53	4.41	0.03	1.11
	Variance	26.12	19.87	244.20	0.02	179.62
F_2	Mean	123.40	13.10	95.10	6.24	41.10
	SE	1.31	0.42	1.02	0.04	1.21
	Min	60.00	8.00	34.00	2.89	16.00
	Max	140.00	28.00	112.3	6.12	94.00
Gemm.-12 (P_4)	Mean	104.12	15.30	72.20	5.26	49.50
	SE	0.53	0.54	0.41	0.06	1.88
	Variance	11.40	28.23	16.22	0.18	167.54
F_1	Mean	113.40	16.30	88.80	3.54	39.40
	SE	0.50	0.53	3.11	0.04	1.53
	Variance	23.15	18.24	232.10	0.03	175.66
F_2	Mean	104.20	11.30	92.20	5.44	39.30
	SE	1.11	0.32	1.23	0.06	1.35
	Min	50.00	6.00	30.00	2.65	15.00
	Max	130.00	25.00	109.20	5.15	92.00

Table (5): Phenotypic (σ_p^2), genotypic (σ_g^2) and environmental (σ_E^2) variances and broad sense heritability (H^2) for the examined traits in F_2 population of the three crosses.

Yield parameters	*Lr*46×Gemmeiza-9				*Lr*46×Gemmeiza-11				*Lr*46×Gemmeiza-12			
	σ_P^2	σ_E^2	σ_g^2	H^2	σ_P^2	σ_E^2	σ_g^2	H^2	σ_P^2	σ_E^2	σ_g^2	H^2
Plant height	304.21	14.32	289.89	71.80	337.03	19.43	317.6	85.70	332.62	17.32	315.3	82.20
No. of spikes/plant	36.35	16.22	20.13	52.10	44.35	19.23	25.12	62.50	41.60	18.45	23.15	59.60
No. of kernels/spike	456.57	119.23	337.34	69.21	476.00	127.22	348.78	80.43	469.68	124.32	345.36	78.32
100 kernel weight	0.45	0.14	0.31	52.45	0.48	0.14	0.34	61.32	0.55	0.13	0.42	59.23
Grain yield/plant	315.77	135.56	180.21	50.11	345.28	146.14	199.14	60.26	333.23	142.12	191.11	59.23

DISCUSSION

The present study included seven wheat cultivars: Gemmeiza-9, Gemmeiza-11, Gemmeiza-12, Misr-1, Misr-2, Sakha-94, and Giza-171, as well as *Lr*46 having different levels of leaf rust severity (%). Furthermore, *Lr*46 can delay the infection procedure or reduce the onset of symptoms brought on by a wider variety of leaf rust races on adult plants, but it does not completely protect the host plant against a particular race of leaf rust (*Puccinia triticina*) (Omara *et al*., 2021). Accordingly, the inheritance and genetic makeup of partial resistance were studied to detect and introgression the slow rusting resistance gene, *Lr*46 in the seven wheat cultivars.

Through the evaluation of seven wheat cultivars at adult stage in the two seasons, the cultivars were divided into two groups; the first included the slow rusting cultivars, namely Misr-1, Misr-2, Sakha-94 and Giza-171. The obtained data showed that there was no segregation in the F_2 plants. Therefore, Misr-1, Misr-2, Sakha-94 and Giza-171 cultivars had the *Lr*46 slow rusting resistance gene. This was explained by Martinez *et al*. (2001), who demonstrated that plants containing *Lr*46 had a considerably shorter latency time than controls without the gene. *Lr*46 also resulted in an increase in the proportion of early-failed fungal colonies. The gene *Lr*46 offers a comparable kind of resistance as *Lr*34, albeit with a less significant impact. William *et al*. (2003) mapped *Lr*46 to the distal end of 1BL using AFLP markers. The researchers discovered that the stripe and leaf rusts resistance genes *Yr*29 and *Lr*46 were pleiotropically related. In the majority of the studied crosses, these findings confirmed partial dominance for reduced disease severity (resistance). Their findings are in agreement with the findings of Abd El-Latif and Boulot (2000) and Hermas and El-Sawi (2015).

The obtained data showed that Gemmeiza-9, Gemmeiza-11 and Gemmeiza-12 cultivars were characterized by fast development of leaf rust (second group). Through genetic analysis, F_2 plants of crosses *Lr*46×Gemmeiza-9 and *Lr*46×Gemmeiza-11 were segregated into 53L: 174H and 47L: 154H. These observed ratios fit the expected ratios of 1:3, and 1:3, respectively. These ratios confirm the presence of one independent recessive gene pair that suppresses leaf rust. Accordingly, the two Gemmeiza cultivars don't carry the slow rust resistance gene, *Lr*46. On the other hand, F_2 plants of cross *Lr*46×Gemmeiza-12 were segregated into 176L: 69H and expected ratio was 3:1. This supporting the hypothesis of one another dominant gene rules the resistance to leaf rust disease in this cross. These results agree with EL-Orabey *et al*. (2020) and Mabrouk *et al*. (2021).

To quantitatively investigate the genetic action of resistance in wheat fields, the parental populations, F_1 and F_2 for each of the three crosses (Gemmeiza-9, Gemmeiza-11, and Gemmeiza-12) with resistance gene *Lr46*, were analyzed for leaf rust in the adult plant. In the three crosses, the F_1 and F_2 plants' mean leaf rust severity values were less severe than those of their respective mid-parents. These findings confirmed the three crosses' partial dominance for disease severity reduction (Abd El-Latif and Boulot 2000 and Hermas and El-Sawi 2015). The expression of the actions of the genes, measured as the degree of dominance h_1 and h_2, was evaluated in three crosses. Significantly negative values of h_1 and h_2 indicated the existence of a partial domain of resistance, while the significantly positive values indicated the existence of an over-dominance of susceptibility.

The variances (S^2) for the parents, F_1's and F_2's in the three crosses were commonly high for each of the tested crosses. The heritability in broad considered to be high in the tested crosses which have high levels of heritability are an

indication of high success rates in restoring desired genes in future generations. These high estimates also suggest that selection for this feature may be achievable during the initial generations of segregation. Given how crucial dominant impacts are to the evolution of this trait, a delay would be more effective (Abd El-Latif and Boulot, 2000; Menshawy and Youssef, 2004; Da-Silva *et al*., 2012; Loladze *et al*., 2014; Hermas and El-Sawi, 2015; EL-Orabey *et al*., 2020 and Mabrouk, *et al*., 2021).

In fact, many researchers believe that the actual number of genes governing rust resistance in wheat is still up for debate. The number of genes controlling such resistance in various wheat genotypes, however, was the subject of inconsistent findings in earlier reports. Several previous studies claimed that wheat rust resistance is a simple hereditary trait governed by one, two, or a small number of gene pairs (Abd El-Latif and Boulot, 2000; Hermas and El-Sawi 2015 and Abdelbacki, *et al*., 2018). Others, however, stress that it is a quantitative trait that is influenced by a variety of gene pairs with additive effects as well as environmental factors (Navabi *et al*., 2005). This finding was corroborated by those data, according to Herrera-Foessel *et al*. (2008), who found that the slow-rust resistance of the durum wheat lines "Playero," "Planeta," and "Trile" was governed by at least three independently inherited genes that interacted in an additive manner. However, this resistance was controlled by at least two genes with cumulative effects in "Piquero," "Amic," "Bergand," "Tagua," and "Knipa".

Given the importance of molecular markers, the results had to be confirmed through them, as the presence of gene *Lr*46 was confirmed in four cultivars, Misr-1, Misr-2, Sakha-94 and Giza-171 and its absence in three cultivars, Gemmeiza-9, Gemmeiza-11, Gemmeiza-12. Also, due to the novelty of these three cultivars, especially Gemmeiza-12, this gene had to be introduced into them in order to give them the characteristic of slow rust resistance. Its introgression was confirmed by molecular markers in first-generation plants (F_2). This saves effort and time as it is possible to rely on molecular marker assisted selection in the early selection of plants carrying this gene (Abdelbacki *et al*., 2014; Abdelbacki *et al*., 2015; Omara and Abdelaal 2017; Abdelbacki, *et al*., 2018 and Omara *et al*., 2021).

Also, due to the importance of the crop characteristics of the three crosses, the plant height, number of spikes per plant, number of kernels per plant, 100-kernel weight, and grain yield per plant were assessed. The mean of F_2 was higher than the average of the parents of the three crosses for yield parameters. The F_2 value ranges for the characters under investigation, also varied from the parents under study. These results demonstrate that the genetic parameters can be calculated due to the sufficient genetic variety of the F_2 generations (Aglan *et al*., 2020).

Plant breeders had to incorporate new efficient resistance genes into their breeding materials because of the dynamic character of the rust infection, which makes it feasible for it to produce new virulent races and makes it possible for it to break down or overcome the host genetic resistance. To take a crucial first step towards the full employment and good exploitation of this resistance in planning and making an appropriate decision in wheat breeding programmes, additional information about the genetic nature and inheritance of rust resistance must thus be made available.

CONCLUSION

Misr-1, Misr-2, Giza-171, and Sakha-94 bread wheat cultivars have the slow rusting resistance gene *Lr*46, according to the qualitative and molecular analysis. While, Gemmeiza-9, Gemmeiza-11, and Gemmeiza-12 cultivars don't carry this gene. As a result, the *Lr46* gene was inserted into these three cultivars to have slow rust resistance, which was verified by molecular markers in F_2 plants. This conserves time and effort because it is easy to choose plants that possess this gene early on using molecular marker-assisted selection. Therefore, plant breeders should take partial resistance into consideration, particularly slow rust resistance genes, rather than depending exclusively on complete rust resistance genes.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest

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