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Molecular markers detection of the leaf rust resistance genes *Lr34*, *Lr74*, *Lr75*, and *Lr80* and their importance for partial resistance in bread wheat genotypes

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Wheat productivity suffers from a severe failure to achieve sufficiency, leading to an increase in global economic need. Leaf rust causes an ongoing issue due to the continuous emergence of new physiological races that break out resistant plant varieties. Molecular-assisted selection technology provides information about slow-rusting genes in the genotype gene pool, which could help the breeding process. The present study aimed to screen fifty genotypes for the slow rusting genes *Lr34*, *Lr74*, *Lr75*, and the new gene *Lr80*. The fifty genotypes were evaluated for leaf rust disease at the Nubaria Research Station during the 2019/20 and 2020/21 seasons. The presence of slow rusting genes was confirmed using a molecular marker tool, which recorded that the highest frequent gene was *Lr74* (86%), and the least frequent gene was *Lr67* (14%). The numbers and combinations of detected slow rusting genes differed from one genotype to another; Giza 168, Misr 3, and BW55213 had the highest observed number of genes among the studied genotypes. We recommend using these genotypes in pyramiding for durable resistance in breeding programs. Partial resistance was assessed simultaneously using the AUDPC, ACI, r-value, CARPA, and RRI parameters. The results of the field evaluation divided the tested genotypes into two main groups: the first group included most of the tested genotypes, which revealed a high level of partial resistance; the second group included Sakha 93, Gemmeiza 7, Gemmeiza 9, and Sids 1, which showed the lowest values of all the parameters and were classified as fast rusting genotypes.

Keywords: Wheat, Leaf rust, Partial resistance, *Lr* genes, Molecular markers

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INTRODUCTION

The world population is expected to reach 9.1 billion; consequently, the need for cereals will increase to more than 3 billion tonnes (Elferink and Schierhorn, 2016). Wheat is considered the most important crop in Egypt, so Egyptian policy has aimed to increase production to 75% of the need for wheat (Fahmi et al., 2015).

Leaf rust is the most challenging and pervasive disease worldwide, caused by *Puccinia triticina* (Gill et al., 2019; Kandiah et al., 2020). The losses in wheat yield resulting from leaf rust infection tend to be less than those due to other wheat rusts. However, the actual losses resulting from leaf rust infection are more regarding the pathogen's ability to recur expansively; therefore, it is assessed as wheat's most destructive rust disease (Bolton et al., 2008; Getie, 2015). Infection decreases the photosynthetic regions, and the pathogen consumes plant nutrients, which results in a decrease in kernel weight per head (Abou-Elseoud et al., 2014), a reduction in overall kernel weight (Bolton et al., 2008; Gill et al., 2019; Sapkota et al., 2019). These losses can be huge in the case of early infection or under suitable climatic conditions. It may reach 20% of the crop yield (Huerta-Espino et al., 2011; Strzembicka et al., 2013), it may reach up to 50% depending on the severity and duration of the infection (K.J. et al., 2018; and McMullen et al., 2008); and the infection diminishes grain quality (Dadrezaei et al., 2013; Figlan et al., 2018). The damage could reduce wheat yields by up to 70 – 80% in susceptible cultivars (Figlan et al., 2018; Hei, 2017). Many cultivars, i.e., Giza 158, Chenab70, SuperX, Giza 139, and Giza 160, have been excluded from the cultivated area in Egypt (Abou-Elseoud et al., 2014). Major epidemics are reported in Australia, New Zealand, and the USA (Murray and Brennan, 2009 and 2010).

Host genetic resistance remains an economical and environmentally friendly approach to diminishing the losses caused by this disease (Dinh et al., 2020; Manjunatha et al., 2018). Accumulating several resistance genes that confer partial resistance in a single genotype is crucial for developing cultivars with more durable rust resistance (Singh and McIntosh, 1992; Singh and Rajaram, 1992), considering a sustainable strategy (Bariana et al., 2007; Dakouri et al., 2013). The average coefficient of infection (ACI), area under disease progress curve (AUDPC), rate of leaf rust disease increase (r-value), and relative resistance index (RRI) parameters were used as practical and trustworthy criteria for genotype evaluation for durable resistance (El-Orabey et al., 2019a and 2020a). This approach helps to ensure that the cultivars in farmers' fields have effective genetic resistance against current rust (Kthiri et al., 2019). Considering the challenges of rust pathogen variation and mutation progress, pathogen genetic variability (Rahmatov et al., 2019; Tomkowiak et al., 2019), and the continuous elimination of cultivars due to susceptibility, the necessity of producing new resistant varieties has increased. Even with the progress of the classical breeding program, it did not meet the requirements for effective cultivars, which could face the development of rust disease. Therefore, knowledge of resistance genes in cultivars can enhance breeding programs (Kazantsev et al., 2019; Rasheed and Xia, 2019).

Resistance of wheat rusts is generally categorized into two nonexclusive types: race-specific and race-nonspecific. Race-specific resistance is generally qualitative and usually short-lived due to the evolution of potentially virulent pathogens resulting from the selection for virulent leaf rust races (Getie, 2015; Huerta-Espino et al., 2020). This resistance class is prone to rapid breakdown as the pathogen population evolves and new virulent races emerge (Carpenter, 2017; Cristina et al., 2015; El-Orabey et al., 2019 b). The other type, non-race specific, is also known as partial resistance (PR); a uniformly effective for almost all pathotypes of the pathogen, it is mainly inherited quantitatively (Herrera-Foessel et al., 2012). Slow rusting resistance is deemed a type of resistance that is both non-specific and durable for both races (Huerta-Espino et al., 2020; Saharan and Ratan, 2011). Slow rusting resistance is characterized by slow epidemic build-up despite a high infection type, indicating a compatible host-pathogen relationship (El-Orabey et al., 2019 b).

Therefore, recent breeding programs have focused on developing adult plant resistance (APR) or slow rusting resistance cultivars. Generally, accumulating APR or slow rusting genes in a single cultivar could lead to close immunity or a high level of resistance of four to five genes in the case of leaf rust (Singh et al., 2011; Singh et al., 2000). Till now, more than 100 *Lr* (Leaf rust) genes have been identified and assigned specific names and symbols (Qureshi et al., 2018; Zhang et al., 2019). Until now, only eight leaf rust resistance genes are known as slow rusting genes, such as *Lr67* (Dyck and Samborski, 1979), *Lr34* (Suenaga et al., 2003), *Lr46* (Rosewarne et al., 2006), *Lr68* (Herrera-Foessel et al., 2012), *Lr74* (McIntosh et al., 2016), *Lr75* (Singla et al., 2017), *Lr77* (Kolmer et al., 2018 a), and *Lr78* (Kolmer et al., 2018 b), which are being pyramided in modern wheat cultivars (Khan and Saini, 2009).

To deal with such a scenario in the future, it is very important to identify leaf rust resistance genes, especially slow-rusting genes, in wheat germplasm to avoid any more leaf rust epidemics (Figlan et al., 2018; Zhang et al., 2019). That is undoubtedly crucial to achieving pyramiding resistance genes in superior cultivars (Ali et al., 2018; Ambrozková et al., 2002); thus, it helps avoid releasing genetically uniform cultivars (Kolmer, 1996). Marker-assisted selection (MAS) improves the efficiency of the selection strategies and provides information about the genetic background of the cultivar (Dakouri et al., 2013; Fahmi et al., 2015; Kazantsev et al., 2019; Adly et al., 2023; Abuzaid; Yousif and Fattah, 2024). The breeder can benefit from molecular marker techniques such as SNPs, STS, SCAR, CAPS, and SSRs that facilitate indirect selection (Ali et al., 2018; Ambrozková et al., 2002). Heterozygosity (H) and polymorphic information content (PIC) were recorded to determine the effectiveness or informativeness of polymorphism as a genetic marker (Alqahtani, 2023). Ahmed et al., (2019) and Elshamy & Mohamed (2022) demonstrated that the PIC relies on the number of alleles and their distribution frequency. The marker index (MI) is a statistical measure calculated to determine the total usefulness of the marker system. Carpenter (2017) and Urbanovich et al., (2006) identified genes *Lr1*, *Lr9*, *Lr10*, *Lr19*, *Lr20*, *Lr21*, *Lr24*, *Lr26*, *Lr34*, *Lr13*, *Lr16*, *Lr25*, *Lr28*, *Lr29*, *Lr35*, *Lr37*, *Lr39*, *Lr46*, *Lr47*, *Lr50*, *Lr51*, and *Lr47* in the cultivars that had not been analyzed for the presence of leaf rust resistance genes; thus, facilitating the pyramiding of unique genes.

Notably, the leaf rust resistance genes *Lr74*, *Lr75*, and the new one *Lr80*, which controls durable resistance, have not been studied in Egyptian plant genotypes until now. On the other hand, the *Lr34* gene was studied in Egyptian varieties, which was detected in this study to confirm its important role in leaf rust resistance. Thus, the present study aimed to evaluate the most Egyptian wheat varieties and lines derived from CIMMYT for leaf rust partial resistance and identify these genes in superior genotypes to enhance wheat breeding to leaf rust resistance with a suitable genome content background.

Table 1. Pedigree of wheat genotypes used in this study.

Code	Genotypes	Pedigree	Origin	Year
G1	Giza 168	MIL/BUC//SERI	Egypt	1999
G2	Giza 171	SAKHA 93 / GEMMEIZA 9	Egypt	2013
G3	Sakha 93	SAKHA 92/TR810328	Egypt	2013
G4	Sakha 94	OPATA/RAYON//KAUZ	Egypt	2004
G5	Sakha 95	PASTOR//SITE/MO/3/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/4/WBLL1	Egypt	2016
G6	Gemmeiza 7	CMH74A.630/SX//SER182/3/AGENT	Egypt	1999
G7	Gemmeiza 9	ALD“S”/HUAC“S”//CMH74A.630/SX	Egypt	1999
G8	Gemmeiza10	MAYA74“S”/0N//160-147/3/BB/GLL/4/CHAT“S”/5/CROW“S”	Egypt	2004
G9	Misr 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR	Egypt	2010
G10	Misr 2	SKAUZ/BAV92	Egypt	2011
G11	Misr 3	ATTILA*2/ABW65*2/KACHU	Egypt	2018
G12	Sids 1	HD2172/PAVON“S”//1158.574“S”	Egypt	1996
G13	Sids 12	BUC//7C/ALD/5/MAYA74/ON//1160-147/3/BB/GLL/4/CHAT“S”/6/MAYA/VUL	Egypt	2007
G14	Sids 13	KAUZ “S”//TSI/SNB“S”	Egypt	2010
G15	Sids 14	SW8488*2/ KUKUNA	Egypt	2018
G16	Shandaweel 1	SITE//MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC.	Egypt	2011
G17	Beni-Suef 5	DIPPERZ/BUSHEN3	Egypt	2007
G18	Beni-Suef 6	BOOMER-21/BUSCA-3	Egypt	2010
G19	BW55751	FRET2*2/BRAMBLING//BECARD/3/WEEBILL1*2/BRAMBLING*2/4/BECARD/QUAIU #1	CIMMYT	2018
G20	BW55144	KACHU//WEEBILL1*2/BRAMBLING*2/3/KACHU/KIRITATI	CIMMYT	2018
G21	BW55619	MUTUS/ROELFS F2007//MUCUY	CIMMYT	2018
G22	BW56959	SUPER 152//PUB94.15.1.12/WEEBILL1/3/MUCUY	CIMMYT	2019
G23	BW50949	BABAX/LR 42//BABAX*2/4/SONOITA F 81/TRAP #1/3/KAUZ*2/TRAP//KAUZ/5/WHEATEAR/SOKOLL	CIMMYT	2014
G24	BW55189	VOROBEY/FISCAL//WEEBILL1*2/KURUKU/3/QUAIU/4/KACHU/KIRITATI	CIMMYT	2018
G25	BW55182	BABAX/LR 42//BABAX*2/3/SHAMA/4/KINGBIRD #1/5/QUAIU/6/2*COPIO	CIMMYT	2018
G26	BW55230	BECARD/QUAIU #1//ONIX/KINGBIRD	CIMMYT	2018
G27	BW55176	ONIX/KINGBIRD*2//KENYA FAHARI/2*KACHU	CIMMYT	2018
G28	BW56961	WEEBILL1//PUB94.15.1.12/WEEBILL1/3/MUCUY	CIMMYT	2019
G29	BW55321	SUPER 152/AKURI//SUPER 152/3/MUCUY	CIMMYT	2018
G30	BW53216	CROC_1/AE.SQUARROSA (205)//BORLAUG M 95/3/PARULA/ICTA SARA 82//TESIA F 79/VEERY #5/4/FRET2/5/TARACHI F 2000/SURUTU-CIAT//KACHU	CIMMYT	2016
G31	BW55173	SUPER 152//WEEBILL1*2/BRAMBLING*2/3/KENYA SWARA/SAUAL//SAUAL	CIMMYT	2018
G32	BW55214	MUTUS*2/KINGBIRD #1/3/KENYA SWARA/SAUAL//SAUAL/4/MUTUS//WEEBILL1*2/BRAMBLING/3/WEEBILL1*2/BRAMBLING	CIMMYT	2018
G33	BW55161	MURGA/KRONSTAD F2004/3/SAUAL/YANAC//SAUAL/6/BABAX/LR 42//BABAX*2/3/KUKUNA/4/CROSBILL #1/5/BECARD	CIMMYT	2018
G34	BW55208	BLOUK #1/KINGBIRD #1*2//BECARD/QUAIU #1	CIMMYT	2018
G35	BW55733	ONIX/KINGBIRD//BORLAUG100 F2014/3/ONIX/KINGBIRD	CIMMYT	2018
G36	BW55654	KACHU/BECARD//WEEBILL1*2/BRAMBLING/3/FRANCOLIN*2/TECUE #1	CIMMYT	2018
G37	BW55177	ONIX/KINGBIRD*2//KENYA FAHARI/2*KACHU	CIMMYT	2018
G38	BW55243	PBW 65/2*PASTOR//SUPER 152/3/CHYAKHURA/4/BECARD/QUAIU #1	CIMMYT	2018
G39	BW55178	ONIX/KINGBIRD*2//KENYA FAHARI/2*KACHU	CIMMYT	2018
G40	BW55193	MUTUS//WEEBILL1*2/BRAMBLING/3/WEEBILL1*2/BRAMBLING/4/KACHU/KINDE	CIMMYT	2018
G41	BW55192	MUTUS//WEEBILL1*2/BRAMBLING/3/WEEBILL1*2/BRAMBLING/4/KACHU/KINDE	CIMMYT	2018
G42	BW55213	K 9644//KIRITATI/2*TARACHI F 2000/3/BECARD/QUAIU #1/4/BABAX/LR 42//BABAX/3/ERA F 2000	CIMMYT	2018
G43	BW55660	WORRAKATTA/2*PASTOR/6/KAUZ/5/PAT10/ALONDRA//PAT72300/3/PAVON F 76/4/BOBWHITE/7/BAJ #1/3/KIRITATI//ATTILA*2/PASTOR	CIMMYT	2018
G44	BW55591	SUPER 152/BAJ #1/4/BAJ #1/3/KIRITATI//ATTILA*2/PASTOR/5/SUPER 152/BAJ #1	CIMMYT	2018
G45	BW55730	KACHU//WEEBILL1*2/BRAMBLING*2/6/ROELFS F2007*2/5/REH/HARE//2*BACANORA T 88/3/CROC_1/AE.SQUARROSA (213)//PAPAGO M 86/4/HUITES F 95	CIMMYT	2018
G46	BW55447	BORLAUG100 F2014*2/3/WEEBILL1*2/TUKURU//CROSBILL #1	CIMMYT	2018
G47	BW56938	SOKOLL/WEEBILL1/5/W15.92/4/PASTOR//HXL7573/2*BAGULA/3/WEEBILL1	CIMMYT	2019
G48	BW56948	PBL94.14.30/4/PASTOR//HXL7573/2*BAGULA/3/WEEBILL1/5/BABAX/LR 42//BABAX/3/ERA F 2000	CIMMYT	2019
G49	BW56949	MEX94.15.34/4/PASTOR//HXL7573/2*BAGULA/3/WEEBILL1/5/BABAX/LR 42//BABAX/3/ERA F 2000	CIMMYT	2019
G50	ACSAD#14	TER-1// MRF1/STJ2/6/ GBY/4/ QUADLETE//ERP/3/UNK/5/TERBOL97-1	ACSAD	2020

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Fifty wheat genotypes (Table 1) were tested for their response to leaf rust at the adult plant stage. Wheat genotypes were provided by the Wheat ResearchDepartment, Field Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt. The experiments were conducted under field conditions at Nubaria Agricultural Research Station (latitude: 30°54'52"N, longitude: 29°58'01"E, elevation: 4 m) during two successive growing seasons, 2019/20 and 2020/21. The planting dates were December 5th and December 1st for the first and second growing seasons. The mean daily temperature and relative humidity exhibited comparable patterns in both years. During May 2020 and 2021, the average maximum air temperatures recorded were 28.91 and 32.39°C, respectively. Between March and May 2020, the daily average temperatures were 16.29 °C, 18.42 °C, and 22.60 °C, respectively. In 2021, during the same period, the average daily temperatures were 15.87°C, 19.01°C, and 25.34°C. The average relative humidity (RH) values from March to May 2020 were 65.68%, 63.56%, and 58.22%, respectively. For the same period in 2021, the average RHs were 65.26%, 57.46%, and 47.08%, respectively (https://power.larc.nasa.gov/data-access-viewer/accessed 20 June 2022); these circumstances were highly suitable for the spread and progression of leaf rust disease (Table 2). The tested wheat genotypes were planted in three replicates with six rows (3.5 m long) 20 cm apart, as each row was sown with 56 g of the wheat as mentioned earlier genotypes. To maintain crop stand/vigor, normal agronomic practices, including recommended fertilization doses, weed control methods, and irrigation schedules, were followed.

Artificial and Field Inoculation

All plants were inoculated at the booting stage, according to the methods of Tarvet and Cassell (1951). The leaf rust urediniospores were obtained from the Wheat Research Diseases Department, Plant Pathology Research Institute, Agricultural Research Center, Egypt. Artificial inoculation was carried out in 75-day-old plants to ensure a threshold of infection. The plants were bordered by a spreader area planted with a mixture of highly susceptible wheat genotypes to leaf rust for field inoculation with leaf rust. These genotypes were Morocco and Thatcher to spread rust inoculum. The plants were treated by spraying them with a mist of water and then dusting them with a mixture of violent urediniospores of the prevalent and strong seven pathotypes, i.e., TTTJT, PTTTT, PTTGS, PTTCT, TTTKT, TTTBT, and TTTTT, mixed with talcum powder at a ratio of 1:20 (v/v) (spore: talcum powder). This process was performed in the early evening (at sunset) before the dew could form on the leaves.

Disease assessment

Leaf rust data were recorded on flag leaves after two weeks of inoculation. The reads were noted at 10-day intervals. Leaf rust disease assessments were carried out using six parameters, as follows: Final leaf rust severity (FRS), average coefficient of infection (ACI), area under disease progress curve (AUDPC), country average relative percentage attack (CARPA), relative resistance index (RRI) and rate of leaf rust disease increase (r-Value). The modified Cobb's scale was used to record FRS for each genotype (Peterson et al., 1948). Plant reaction (infection type) was classified into five categories (Stakman et al., 1962): immune (0), resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S). The coefficient of infection (CI) was calculated, according to Saari & Wilcoxson (1974) and Pathan & Park (2006), by multiplying rust severity with certain constant values assigned to each infection type (IT). The constant values for the different infection types were as follows: R = 0.2, MR = 0.4, MS = 0.8, and S = 1 (Stubbs et al., 1986). The ACI was calculated by adding the CI values for each line and dividing the sum by the total number of seasons. To calculate the country's average relative percentage attack (CARPA), the candidate line with the highest ACI is assigned a value of 100; all other lines are adjusted proportionally. The numerical scale earlier identified as the resistance index (RI), ranging from 0 to 9, has been reclassified and referred to as the relative resistance index (RRI). From CARPA, the value of RRI is determined on a 0 to 9 scale, where 0 represents the most susceptible and 9 indicates highly resistant (Aslam, 1982; Akhtar et al., 2002). The recommended index score for leaf rust resistance is seven or above, while 6 or 5 is still acceptable (Aslam, 1982). The formula used to compute the RRI is as follows:

$$\mathrm{RRI} = \frac{100 - \mathrm{CARPA}}{100} \quad * 9$$

AUDPC was assessed to compare different responses of the tested genotypes to leaf rust. It was calculated using FRS and CI, as Pandey et al., (1989) described.

$$\mathrm{AUDPC} = D\,[1/2\,(Y1 + Yk) + (Y2 + Y3 + \ldots\ldots + Y(k\text{-}1)]$$

Where:

D = refers to the number of days between two successive records, which can also be described as time intervals.

Y1 + Yk = The sum of the initial and final disease scores.

Y2 + Y3 + ... + Yk-1 = the sum of all disease scores between the first and last scores.

The rate of leaf rust increase (r-value) was estimated to assess the capability of the tested genotype to affect the development of wheat leaf rust infection. It was calculated by measuring the severity of the infection at the time rust pustules appeared, and every seven days, the following formula was assumed by Plank (1963):

$$\mathrm{r-value} = \frac{1}{\mathrm{t}_2 - \mathrm{t}_1} * \left(\log\frac{\mathrm{x}_2}{1-\mathrm{x}_2} - \log\frac{\mathrm{x}_1}{1-\mathrm{x}_1}\right)$$

All leaf rust disease assessment parameters were recorded for three replicates, and the means of the replicate data were calculated.

Molecular detection of *Lr* Genes

PCR detection and DNA isolation were conducted at the Nucleic Acids Research Department Labs, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt.

DNA Extraction: DNA of the fifty plant genotypes was isolated from green leaves during the seedling stage using an EZ-Spin Column Genomic Plant DNA Extraction, DNA Miniprapa Kit (Bio Basic INC, New York, USA), according to the manufacturer's instructions. The isolated DNA concentration was measured, and DNA quality was calculated using BioDropµLITE (BioDrop, Cambridge, England) at 260 and 280 nm wavelengths.

PCR Amplification and Gel Analysis: Specific markers were used to verify the presence of four *Lr* genes, *Lr34*, *Lr74*, *Lr75*, and *Lr80*, by using four specific primer pairs in wheat genotypes, and the information about these markers, including their names, sequences, fragment sizes, annealing temperatures, and references, are listed in Table 3. Whereas data from previously studied *Lr46*, *Lr67*, and *Lr68* were obtained from (https://wgb.cimmyt.org/gringlobal/search) and EL-Oraby et al., 2019 a. These four genes were screened in the Egyptian genotypes. The PCR amplification was achieved using the Qiagen Taq PCR Master Mix Kit (Qiagen, Santa Clarita, CA, USA) and the T100TM Thermal Cycler (Bio-Rad, Singapore). The PCR reaction mixture (25 µL) contained 30 ng of DNA template and ten pmol of each forward and reverse primer. The reaction conditions were as follows: initial denaturation was for 5 min at 94°C, followed by 35 cycles of denaturation for 5 min at 94°C, 30-sec annealing for (50 – 60) °C followed by extension at 72°C for 2 min; subsequently, a 7 min final extension at 72°C was done. The PCR products were separated by electrophoresis on 3% agarose gel in TBE buffer (45 mM Tris-borate, one mM EDTA, pH 8). The bands were visualized using a gel documentation system (Syngene, UK). A 50 bp DNA Ladder RTU (Gene Direx, Bio Innovation, Germany) was used to determine the size of the amplification fragments. The amplified bands were scored as present (1) or absent (0) to create the binary dataset across the 50 genotypes for each primer.

Data analysis

Field tests were conducted in a randomized complete block design (RCBD) with three replicates. A combined analysis of variance over the two seasons was carried out using a statistical analysis system (version 9.2. SAS Institute, Inc., Cary, NC, U.S.A.) (Table 4). The significance of differences among the studied genotypes was tested by analysis of variance (ANOVA) as outlined by Snedecor and Cochran (1967). The means of all studied traits for the fifty genotypes across two years were compared using the Fisher's least significant difference (LSD) test at P = 0.05 (Sokal and Rohlf, 1981). A cluster analysis of the tested genotypes against leaf rust disease was applied to the data of the area under the disease progress curve (AUDPC) over the two seasons, as well as the data obtained from the detection of the *Lr* genes under study. A dendrogram based on the unweighted pair group method with arithmetic mean (UPGMA) was also constructed with PAST 4.12 software (Hammer et al., 2001). Correlation analysis between leaf rust disease assessment parameters and the number of *Lr* genes within each tested wheat genotype was conducted using correlation matrix online software (http://www.sthda.com/english/rsthda/correlation-matrix.php).

RESULTS

Evaluation of wheat genotypes against leaf rust under field conditions

The response of wheat genotypes against *P. triticina* at the adult stage in fifty wheat genotypes is recorded as the final leaf rust severity in Table 5. The obtained

Table 2. Monthly weather averages in the Nubaria region during the 2019/20 and 2020/21 growing seasons.

	TMIN (°C)		TMAX (°C)		TAVE (°C)		RAIN (mm)		R.H. %		WIND (km/h)	
Month	19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21
December	12.53	12.86	20.30	21.30	16.42	17.08	0.92	0.07	67.73	66.58	13.90	10.19
January	10.12	11.24	17.05	19.93	13.59	15.59	1.82	0.39	70.80	69.14	13.93	10.98
February	10.17	10.39	18.58	19.97	14.38	15.18	1.04	1.07	70.92	66.60	11.56	13.18
March	11.02	11.24	21.56	20.50	16.29	15.87	2.16	9.58	65.68	65.26	13.14	15.12
April	12.98	12.37	23.85	25.64	18.42	19.01	2.06	0.03	63.56	57.46	11.02	13.28
May	16.30	18.29	28.91	32.39	22.60	25.34	0.00	0.02	58.22	47.08	12.35	17.03

TMIN: minimum temperature; TMAX: maximum temperature; TAVE: daily average temperature; RAIN: precipitation; R.H: relative humidity; WIND: wind speed.

Table 3. PCR primers were used to identify the four slow-leaf rust resistance genes in the wheat genotypes.

Gene	Marker	Sequence of primers 5'- 3'	Fragment Size (bp)	Annealing Temperature (°C)	Type	References
Lr34	csLV34	F: GTTGGTTAAGACTGGTGATGG	150	55	STS	Lagudah et al., (2006)
		R: TGCTTGCTATTGCTGAATAGT				
Lr74	xgwm533	F: AAGGCGAATCAAACGGAATA	120	60	SSR	Li et al., (2017)
		R: GTTGCTTTAGGGGAAAAGCC				
Lr75	swm271	F: GTCCATTCGGCGCTAGATCG	200	50	SSR	Singla et al., (2017)
		R: CTGGCTCCGGCACCTTATCA				
Lr80	barc124	F: TGCACCCCTTCCAAATCT	260	52	SSR	Kumar et al., (2021)
		R: TGCGAGTCGTGTGGTTGT				

Table 4. ANOVA for leaf rust severity of 50 genotypes evaluated in Nubaria location during 2019/20 and 2020/21.

S.O.V	df	Mean Square									
		ACI	p-value	AUDPC	p-value	CARPA	p-value	RRI	p-value	r-Value	p-value
G	49	1111.42^{**}	0.000	110085.28^{**}	0.000	1372.17^{**}	0.000	11.11^{**}	0.000	0.00239^{**}	0.000
S	1	7723.54^{**}	0.000	550943.02^{**}	0.000	9534.42^{**}	0.000	77.21^{**}	0.000	0.03480^{**}	0.000
G * S	49	655.72^{**}	0.000	56075.78^{**}	0.000	809.55^{**}	0.000	6.56^{**}	0.000	0.00121^{**}	0.000
Error	196	85.78		8316.05		105.91		0.86		0.00017	

S.O.V. Source of variation; G.Genotype; S.Seasons; ** Highly significant; ACI.Average coefficient of infection; AUDPC.Area under disease progression curve; CARPA.Country average relative percentage attack; RRI.Relative resistance index; r-Value rate of leaf rust disease increase.

records revealed a range of response levels of the tested wheat genotypes to leaf rust disease during both growing seasons. Fifty wheat genotypes showed different reactions among the two growing seasons: final leaf rust severity varied from R (resistant) to MR (moderately resistant) for nine genotypes in two seasons: Sakha 94, Sakha 95, Sids 12, BW55751, BW50949, BW55230, BW56961, BW55161, and BW55243. Fourteen genotypes recorded susceptible reactions in two growing seasons, ranging from Tras MS (moderately susceptible) to 80 S (susceptible), except Beni-Suef 5, which revealed MR/MS reactions in the second season. The rest of the tested genotypes gave different responses in two seasons; most gave an R reaction in the first year and then a susceptible reaction in the second season. The infection rate was higher in the second season than in the first season.

Evaluation of wheat genotypes for partial leaf rust resistance:

Analysis of the variance of values of the ACI, AUDPC, CARPA, r-value, and RRI parameters for the tested wheat genotypes showed that the effects of genotype, environment, and the interaction between the two genotypes on the leaf rust infection response were highly significant (Table 4). In the first growing season of 2019/20, the ACI of the tested genotypes ranged from 0.6% to 50%, whereas in the second season of 2020/21, the values ranged from 0.6% to 80% at the Agricultural Research Station in Nubari (Table 6). Thus, all the tested genotypes can be classified into two groups based on the mean ACI (Table 6) during two growing seasons, according to (Draz et al., 2015). The first group had ACI values up to 20%, revealing partial resistance: Giza 168, Gemmeiza 10, Misr 1, Misr 3, Sakha 94, Sakha 95,

Table 5. Leaf rust severity of 50 wheat genotypes at the Nubaria location during the growing season (2019/2020, 2020/2021).

Code	Genotypes	2019/2020	2020/2021
G1	Giza 168	10MR	10S
G2	Giza 171	10S	40S
G3	Sakha 93	10S	70 S
G4	Sakha 94	Tras MR	Tras MR
G5	Sakha 95	5MR	Tras R
G6	Gemmeiza 7	15S	70S
G7	Gemmeiza 9	10S	70S
G8	Gemmeiza 10	10S	30S
G9	Misr 1	5S	10MR
G10	Misr 2	5MS	15MS
G11	Misr 3	Tras MR	Tras MS
G12	Sids 1	40S	80S
G13	Sids 12	5R	Tras MR
G14	Sids 13	5MS	10S
G15	Sids 14	5R	40MS
G16	Shandaweel 1	10MR	20S
G17	Beni-Suef 5	40S	5MR/MS
G18	Beni-Suef 6	50S	10S
G19	BW55751	5R	Tras R
G20	BW55144	Tras R	15S
G21	BW55619	5R	20S
G22	BW58064	Tras MR	5S
G23	BW50949	5R	Tras MR
G24	BW55189	Tras R	Tras S
G25	BW55182	Tras R	Tras S
G26	BW55230	Tras R	Tras R
G27	BW55176	Tras R	10S
G28	BW56961	Tras R	Tras MR
G29	BW55321	Tras R	5MS
G30	BW53216	5 R	5MS
G31	BW55173	10MR	Tras MR/MS
G32	BW55214	Tras MR	20S
G33	BW55161	Tras R	5R
G34	BW55208	Tras R	40S
G35	BW55733	Tras MS	5 MS
G36	BW55654	10R	5MS
G37	BW55177	5MR	Tras S
G38	BW55243	Tras R	Tras MR
G39	BW55178	Tras MR	20S
G40	BW55193	Tras R	20S
G41	BW55192	Tras R	40S
G42	BW55213	Tras R	20MS
G43	BW55660	10R	10S
G44	BW55591	Tras R	20S
G45	BW55730	Tras R	Tras MS
G46	BW55447	5 R	20S
G47	BW56938	10MS	20S
G48	BW56948	20S	5 S
G49	BW56949	15MR	5S
G50	ACSAD#14	30S	20S

FRS, final leaf rust severity; MR, moderately resistant; MS, moderately susceptible; S, susceptible; R, resistant; Tras, < 5%; ACSAD, the Arab Center for Studies of Arid Zones and Arid Lands.

Shandaweel 1, Sids 12, Sids 13, Sids 14, and all lines except ACSAD#14, the second group recorded ACI values more than 20% showing fast rusting: Beni-Suef 5, Beni-Suef 6, Sids 1, Gemmeiza 7, Gemmeiza 9, Sakha 93, Giza 171, and ACSAD#14. Additionally, in the first season, all the tested wheat genotypes showed a desirable or acceptable RRI ranging from 6.00 to 8.94, except Beni-Suef 5, Sids 1, and Beni-Suef 6 showed 5.00, 5.00, and 4.00, respectively. Whereas in the second season, most of the tested wheat genotypes showed desirable/acceptable RRI ranging from 5.80 to 8.94, except eight wheat genotypes, i.e., Giza 171 (5.00), Sakha 93 (2.00), Gemmeiza 7 (2.00), Gemmeiza 9 (2.00), Sids 1 (1.00), BW55208 (5.00), and BW55192 (5.00) (Table 6).

Moreover, the area under disease progress curve (AUDPC) values during the 2019/2020 and 2020/2021 growing seasons ranged from 3.15 to 318.24 in the first season and from 6.25 to 953.78 in the second season (Table 6). In the two growing seasons, most of the tested genotypes had the lowest AUDPC values (less than 332.5), indicating that these genotypes exhibited partial resistance. On the other hand, only one genotype, Sids 1, recorded more than 332.5 in two growing seasons. While in the second season, six genotypes recorded more than 332.5: Giza 171 (333.57), Sakha 93 (591.27), Gemmeiza 7 (757.12), Gemmeiza 9 (842.08), Sids 1 (953.78), Sids 14 (362.26), and BW55192 (310.60), are grouped in two classes according to (Draz et al., 2015; and El-Orabey et al., 2019a) (Table 6). These results were obtained by a dendrogram constructed based on AUDPC, as shown in Figure 1. This cluster is divided into two main groups: partial resistant genotypes and fast-rusting genotypes.

The Country Average Relative Percentage Attack (CARPA) values for the 50 wheat genotypes during the 2019/20 and 2020/21 growing seasons were assessed. The CARPA values provide insights into the relative percentage attack of leaf rust disease across the tested genotypes. The CARPA values ranged from 0.67 to 55.56 with an average of 6.81 in the first season and from 0.67 to 88.89 with an average of 17.72 in the second season (Table 6), indicating the varying levels of susceptibility to leaf rust among the genotypes. Most of the tested genotypes exhibited lower CARPA values, suggesting a higher partial resistance to leaf rust.

In addition, the genotypes were classified into two groups based on second season data of the r-value according to (Draz et al., 2015). The first group of genotypes recorded an r-value of more than 0.101, including Sakha 93, Gemmeiza 7, Gemmeiza 9, and Sids 1, considered fast-rusting genotypes. The second group recorded an r-value up to 0.101, showing partial resistance. These groups included all genotypes except the genotypes in the first group above.

The correlation between partial resistance parameters and *Lr* gene content:

The correlations among the partial resistance parameters ACI, AUDPC, the RRI, the r-value, and the slow rusting gene content number in genotypes were recorded. It was found that there was a negative correlation between AUDPC, ACI, r-value parameters, and slow rusting genes content. In contrast, the RRI parameter was positively correlated with the slow rusting genes content. (Figure 2).

Molecular detection of slow rusting genes by using closely linked SSR markers

The leaf rust resistance genes *Lr34*, *Lr74*, and *Lr75* and the new gene *Lr80*, which controls durable leaf rust resistance, were identified in fifty wheat genotypes using molecular markers as follows:

Molecular detection of the *Lr34* gene: The primers of the csLV34 STS marker amplified two fragments of 150 and 229 bp. The positive 150 bp fragment was amplified in ten wheat genotypes: Sakha 94, Sakha 95, Sids 13, Misr 3, Shandaweel 1, BW55193, BW55660, BW55591, BW55208, and BW55730 (Figure 3A), indicating that these genotypes have the leaf rust resistance gene *Lr34* (Table 7). While the other tested genotypes showed the 229 bp fragment, indicating the absence of *Lr34* in these wheat genotypes.

Molecular detection of *Lr74* gene: The Xgwm533-3B SSR marker was confirmed to be used in MAS for the *Lr74* gene. The electrophoretic pattern in Figure 3B showed SSR-specific and polymorphic bands representing 120 bp fragments in forty-three genotypes, indicating that these genotypes possess the *Lr74* gene (Table 7).

Molecular detection of *Lr75* gene: The *Lr75* gene is a novel partial adult plant leaf rust resistance gene. The Swm271 SSR marker was used to screen all genotypes for the *Lr75* gene. The electrophoretic pattern in Figure 3C showed SSR-specific and polymorphic bands representing over 200 bp fragments in twenty-seven genotypes, indicating that these genotypes possess the *Lr75* gene (Table 7).

Molecular detection of *Lr80* gene: The Barc124 SSR marker has recently been used for marker-assisted selection of the *Lr80* gene, which reveals successful pyramiding with other genes to confer durable leaf rust resistance. The amplicon sizes among genotypes varied from 264 to 270 bp, which represents the existence of the *Lr80* gene in 28 tested genotypes (Figure 3D). In addition, the results of slow rusting genes detected in the tested fifty genotypes (*Lr46*, *Lr67*, and *Lr68*) from previous research were presented in the present study (Table 7). This was done to count the number of slow rusting genes in each genotype and determine their combined effects on leaf rust resistance.

The analysis of marker efficiency targeting leaf rust slow rusting genes delineated discernible parameter values across all primers, as detailed in Table 8. The molecular characterization of the four *Lr* tested genes yielded five detectable bands/amplicons. The polymorphism rate (PR) remained consistently observed at 100% for all primers. Heterozygosity (H) manifested values ranging from 0.2418 (*Lr74*) to 0.4978 (*Lr75*) across the primers. Likewise, polymorphism information content (PIC) varied between 0.2128 (*Lr74*) and 0.3733 (*Lr75*). The effective multiplex ratio (E) spanned from 0.2 (*Lr34*) to 0.86 (*Lr74*). The arithmetic mean of H (H.av) spanned from 0.00581 (*Lr74*) to 0.01093 (*Lr75*). The marker index (MI) ranged from 0.001280 (*Lr34*) to 0.005519 (*Lr80*). Discriminating power (D) values ranged from 0.262857 (*Lr74*) to 0.963265 (*Lr34*). The resolving power (R) values ranged from 0.28 (*Lr74*) to 0.92 (*Lr75*). *Lr75* exhibited the highest H, H.av, R, and PIC values, while *Lr34* showed the lowest E and MI values but maintained the highest D value. *Lr74* demonstrated comparatively lower H, PIC, H.av, D, and R values in contrast to other *Lr* genes yet revealed the highest E value. *Lr80* showcased the highest MI value.

The molecular phylogeny analysis (Figure 4) based on the *Lr* gene divided the genotypes depending on the content of *Lr* genes in each genotype. However, the investigated genotypes were not divided into groups according to the levels of partial resistance to leaf rust based on the AUDPC parameter.

Based on the results of molecular detection of *Lr* genes, it was found that the *Lr34* gene was found in 20 % of genotypes, *Lr46* in 62%, *Lr67* in 14%, *Lr68* in 38 %, *Lr74* in 86%, *Lr75* in 54%, and *Lr80* in 56%, respectively. In the present study, the tested genotypes showed different combinations of resistance genes (Table 7) and could be divided into seven groups based on the number of tested slow-rusting genes. The first group indicated the absence of all tested genes in G50, G49, and G48. The genotypes

Table 6. Average coefficient of infection (ACI), area under disease progress curve (AUDPC), Country average relative percentage attack (CARPA), Relative resistance index (RRI), and Rate of leaf rust disease increase (r-Value) of 50 wheat genotypes at Nubaria location during growing seasons (2019/2020, 2020/2021).

Code	Genotypes	ACI		AUDPC		CARPA		RRI		r-Value	
		19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21
G1	Giza 168	4.00	10.00	34.70	48.97	4.44	11.11	8.60	8.00	0.035	0.058
G2	Giza 171	10.00	40.00	71.05	333.57	11.11	44.44	8.00	5.00	0.051	0.086
G3	Sakha 93	10.00	70.00	119.25	591.27	11.11	77.78	8.00	2.00	0.061	0.105
G4	Sakha 94	1.20	1.20	14.65	12.38	1.33	1.33	8.88	8.88	0.028	0.036
G5	Sakha 95	2.00	0.60	22.58	12.15	2.22	0.67	8.80	8.94	0.038	0.011
G6	Gemmeiza 7	15.00	70.00	255.70	757.12	16.67	77.78	7.50	2.00	0.067	0.114
G7	Gemmeiza 9	10.00	70.00	116.66	842.08	11.11	77.78	8.00	2.00	0.06	0.119
G8	Gemmeiza 10	10.00	30.00	50.18	264.05	11.11	33.33	8.00	6.00	0.062	0.086
G9	Misr 1	5.00	4.00	90.46	32.02	5.56	4.44	8.50	8.60	0.053	0.051
G10	Misr 2	4.00	12.00	81.33	108.60	4.44	13.33	8.60	7.80	0.052	0.06
G11	Misr 3	1.20	2.40	9.21	26.90	1.33	2.67	8.88	8.76	0.031	0.039
G12	Sids 1	40.00	80.00	318.24	953.78	44.44	88.89	5.00	1.00	0.079	0.129
G13	Sids 12	1.00	1.20	16.80	6.25	1.11	1.33	8.90	8.88	0.023	0.032
G14	Sids 13	4.00	10.00	27.20	56.62	4.44	11.11	8.60	8.00	0.043	0.065
G15	Sids 14	1.00	32.00	22.56	362.26	1.11	35.56	8.90	5.80	0.029	0.103
G16	Shandaweel 1	4.00	20.00	60.43	236.32	4.44	22.22	8.60	7.00	0.043	0.084
G17	Beni-Suef 5	40.00	3.00	295.00	33.08	44.44	3.33	5.00	8.70	0.085	0.036
G18	Beni-Suef 6	50.00	10.00	300.55	61.50	55.56	11.11	4.00	8.00	0.089	0.053
G19	BW55751	1.00	0.60	16.33	15.97	1.11	0.67	8.90	8.94	0.029	0.019
G20	BW55144	0.60	15.00	5.79	85.28	0.67	16.67	8.94	7.50	0.016	0.074
G21	BW55619	1.00	20.00	13.20	119.95	1.11	22.22	8.90	7.00	0.033	0.081
G22	BW58064	1.20	5.00	20.76	28.17	1.33	5.56	8.88	8.50	0.028	0.046
G23	BW50949	1.00	1.20	22.37	11.35	1.11	1.33	8.90	8.88	0.035	0.036
G24	BW55189	0.60	3.00	9.05	19.32	0.67	3.33	8.94	8.70	0.022	0.05
G25	BW55182	0.60	3.00	7.60	18.97	0.67	3.33	8.94	8.70	0.022	0.038
G26	BW55230	0.60	0.60	6.41	8.38	0.67	0.67	8.94	8.94	0.019	0.007
G27	BW55176	0.60	10.00	9.23	64.53	0.67	11.11	8.94	8.00	0.023	0.058
G28	BW56961	0.60	1.20	4.60	11.35	0.67	1.33	8.94	8.88	0.013	0.036
G29	BW55321	0.60	4.00	6.05	40.97	0.67	4.44	8.94	8.60	0.013	0.052
G30	BW53216	1.00	4.00	7.81	30.60	1.11	4.44	8.90	8.60	0.026	0.047
G31	BW55173	4.00	1.80	27.46	13.42	4.44	2.00	8.60	8.82	0.045	0.023
G32	BW55214	1.20	20.00	10.66	195.27	1.33	22.22	8.88	7.00	0.031	0.076
G33	BW55161	0.60	1.00	5.32	21.97	0.67	1.11	8.94	8.90	0.013	0.028
G34	BW55208	0.60	40.00	7.13	219.05	0.67	44.44	8.94	5.00	0.019	0.079
G35	BW55733	2.40	4.00	14.86	27.12	2.67	4.44	8.76	8.60	0.04	0.036
G36	BW55654	2.00	4.00	18.59	24.02	2.22	4.44	8.80	8.60	0.039	0.05
G37	BW55177	2.00	3.00	19.52	18.30	2.22	3.33	8.80	8.70	0.035	0.037
G38	BW55243	0.60	1.20	8.56	8.83	0.67	1.33	8.94	8.88	0.023	0.025
G39	BW55178	1.20	20.00	9.93	216.72	1.33	22.22	8.88	7.00	0.024	0.074
G40	BW55193	0.60	20.00	5.32	75.58	0.67	22.22	8.94	7.00	0.013	0.062
G41	BW55192	0.60	40.00	5.32	310.60	0.67	44.44	8.94	5.00	0.013	0.088
G42	BW55213	0.60	16.00	3.15	72.42	0.67	17.78	8.94	7.40	0	0.052
G43	BW55660	2.00	10.00	12.97	46.90	2.22	11.11	8.80	8.00	0.036	0.05
G44	BW55591	0.60	20.00	7.86	133.20	0.67	22.22	8.94	7.00	0.019	0.075
G45	BW55730	0.60	2.40	5.69	14.38	0.67	2.67	8.94	8.76	0.019	0.042
G46	BW55447	1.00	10.00	9.00	60.58	1.11	11.11	8.90	8.00	0.026	0.07
G47	BW56938	8.00	20.00	40.78	138.93	8.89	22.22	8.20	7.00	0.05	0.075
G48	BW56948	20.00	5.00	164.03	26.62	22.22	5.56	7.00	8.50	0.072	0.053
G49	BW56949	6.00	5.00	56.39	35.75	6.67	5.56	8.40	8.50	0.053	0.044
G50	ACSAD # 14	30.00	20.00	292.08	102.25	33.33	22.22	6.00	7.00	0.078	0.08
Mean	-	6.13	15.95	55.21	139.11	6.81	17.72	8.39	7.41	0.04	0.06
LSD of (G) at 5%	-	10.55		103.83		11.72		1.05		0.015	
LSD of (S) at 5%	-	2.11		20.77		2.34		0.21		0.003	

ACI Average coefficient of infection; AUDPC Area under disease progress curve; CARPA Country average relative percentage attack; RRI Relative resistance index; r-Value Rate of leaf rust disease increase; G Genotypes; S Season.

Table 7. Presence and absence of tested slow rusting genes in the present study and those previously reported within the 50 wheat genotypes.

Code	Genotypes	*Lr34*	*Lr46*	*Lr67*	*Lr68*	*Lr74*	*Lr75*	*Lr80*	No. *Lr*
G1	Giza 168	-	+	+	+	+	+	+	6
G2	Giza 171	-	+	-	-	+	-	-	2
G3	Sakha 93	-	-	-	-	+	-	-	1
G4	Sakha 94	+	-	-	-	+	-	-	2
G5	Sakha 95	+	-	-	-	+	-	+	3
G6	Gemmeiza 7	-	-	+	-	+	-	-	2
G7	Gemmeiza 9	-	+	-	-	+	-	+	3
G8	Gemmeiza 10	-	+	-	-	+	-	-	2
G9	Misr 1	-	-	+	+	+	+	+	5
G10	Misr 2	-	-	+	+	+	+	-	4
G11	Misr 3	+	-	+	+	+	+	+	6
G12	Sids 1	-	-	-	-	+	+	+	3
G13	Sids 12	-	+	-	-	+	+	-	3
G14	Sids 13	+	-	-	-	+	-	-	2
G15	Sids 14	-	-	-	-	+	-	-	1
G16	Shandaweel 1	+	-	-	-	+	-	+	3
G17	Beni-Suef 5	-	-	-	-	+	+	+	3
G18	Beni-Suef 6	-	-	-	-	-	+	+	2
G19	BW55751	-	+	-	+	+	-	-	3
G20	BW55144	-	+	-	+	-	+	+	4
G21	BW55619	-	+	-	-	+	-	+	3
G22	BW58064	-	+	-	-	-	+	+	3
G23	BW50949	-	+	-	-	+	-	-	2
G24	BW55189	-	+	-	+	+	-	-	3
G25	BW55182	-	+	-	+	+	+	+	5
G26	BW55230	-	+	-	+	+	-	-	3
G27	BW55176	-	+	-	+	+	-	-	3
G28	BW56961	-	-	+	-	+	+	+	4
G29	BW55321	-	+	-	-	-	+	+	3
G30	BW53216	-	+	-	-	+	+	+	4
G31	BW55173	-	+	-	+	+	+	+	5
G32	BW55214	-	+	-	-	+	+	+	4
G33	BW55161	-	+	-	+	+	-	-	3
G34	BW55208	+	+	-	-	+	+	+	5
G35	BW55733	-	-	-	-	+	-	+	2
G36	BW55654	-	+	-	+	+	+	+	5
G37	BW55177	-	+	-	+	+	+	+	5
G38	BW55243	-	+	-	+	+	+	+	5
G39	BW55178	-	+	-	+	+	+	+	5
G40	BW55193	+	+	-	-	+	+	-	4
G41	BW55192	-	+	-	-	+	+	+	4
G42	BW55213	-	+	+	+	+	+	+	6
G43	BW55660	+	+	-	+	+	-	-	4
G44	BW55591	+	+	-	-	+	+	-	4
G45	BW55730	+	+	-	+	+	-	+	5
G46	BW55447	-	+	-	-	+	+	+	4
G47	BW56938	-	-	-	-	+	+	-	2
G48	BW56948	-	-	-	-	-	-	-	0
G49	BW56949	-	-	-	-	-	-	-	0
G50	ACSAD#14	-	-	-	-	-	-	-	0

Table 8. Marker efficiency analysis of leaf rust slow rusting genes primers.

Primer	NAB	NMB	NPB	PR%	TB	H	PIC	E	H.av	MI	D	R
Lr34	2	0	2	100	10	0.3200	0.2698	0.20	0.00640	0.001280	0.963265	0.40
Lr74	1	0	1	100	43	0.2418	0.212808	0.86	0.005816	0.004142	0.262857	0.28
Lr75	1	0	1	100	27	0.4978	0.373395	0.54	0.010936	0.005365	0.713469	0.92
Lr80	1	0	1	100	28	0.4938	0.371374	0.56	0.010856	0.005519	0.691429	0.88

NAB, No. of Amplified bands; NMB, No. of Monomorphic bands; NPB, No. of Polymorphic bands; PR, Polymorphism rate; TB, Total bands; H, heterozygosity index; PIC, polymorphism information content; E, effective multiplex ratio; H.av, arithmetic mean of H; MI Marker Index; D discriminating power; R, resolving power

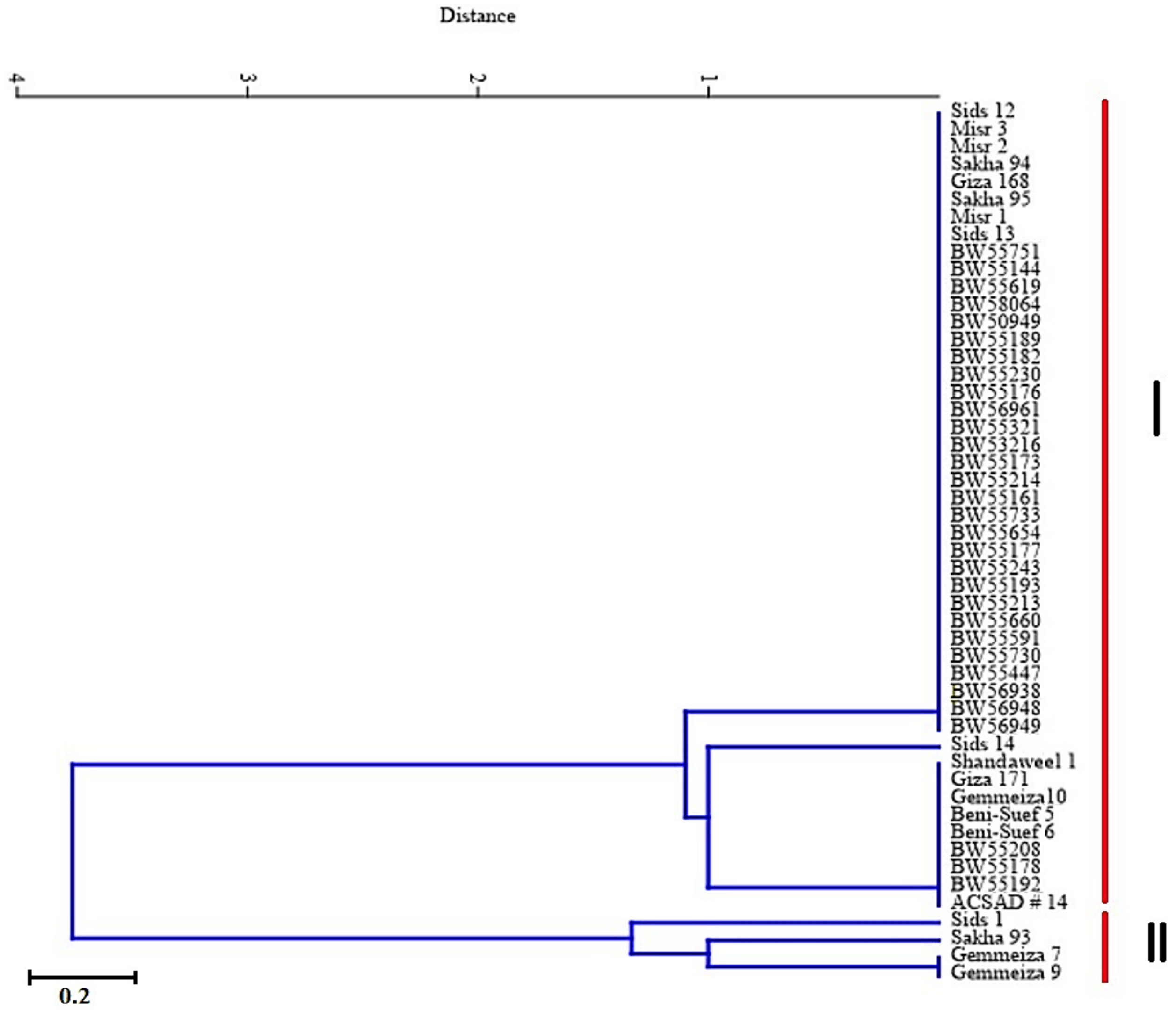

Figure 1. Cluster analysis of 50 wheat genotypes based on the AUDPC assessed under field conditions during 2019/2020–2020/2021

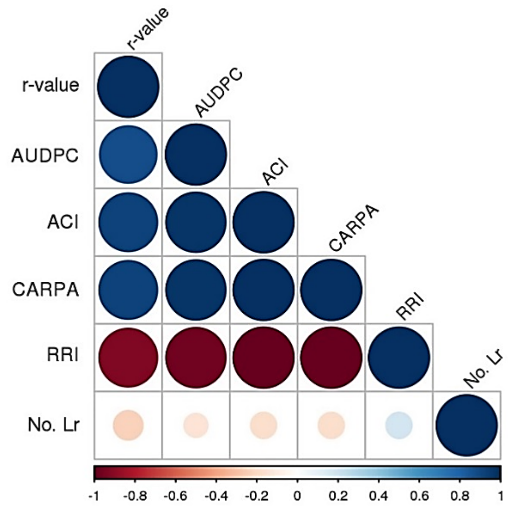

Figure 2. Correlations among ACI, AUDPC, CARPA, the RRI, the r value, and the number of slow rusting gene content number in genotypes.

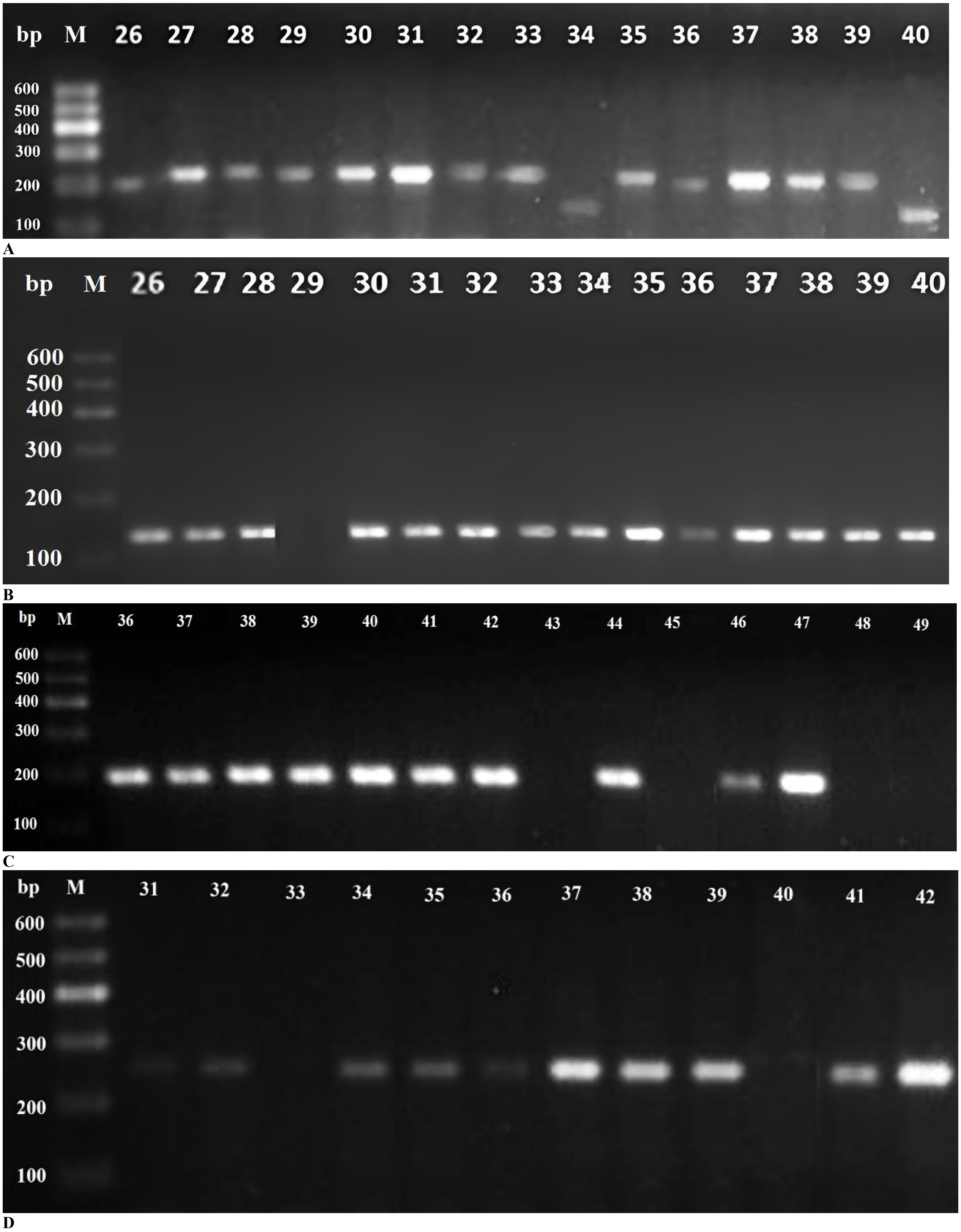

Figure 3: Amplification products using specific markers for genes *Lr34* (A), *Lr74* (B), *Lr75* (C), and *Lr80* (D) respectively in the studied wheat genotypes.

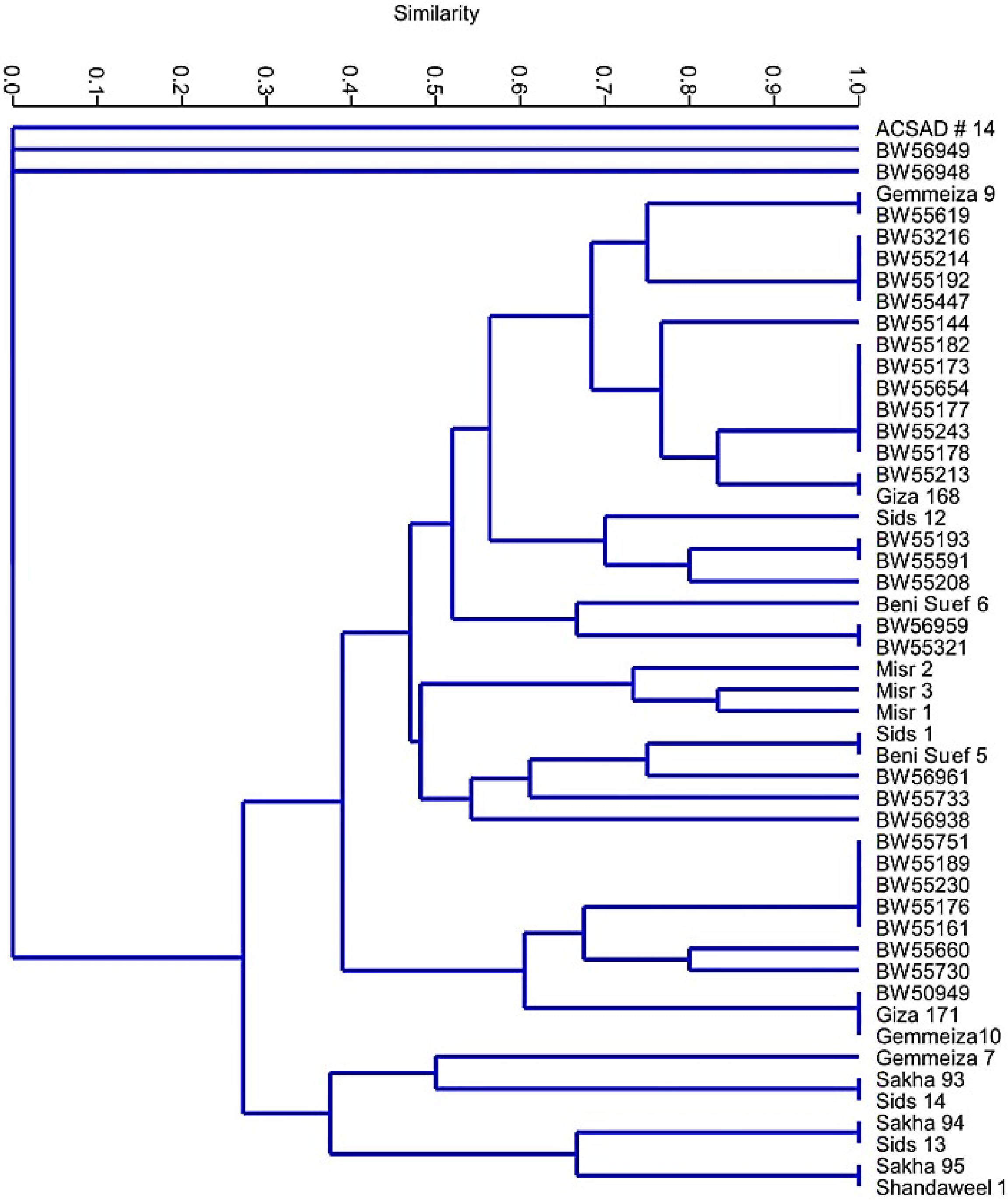

Figure 4. Phylogenetic tree showing the similarity among 50 wheat varieties based on Jaccard's similarity analysis of four *Lr* gene markers.

in this group have low to moderate AUDPC values and susceptible final disease severity (Tables 5 and 6) except G49, recorded as MR in the first season. The second group includes genotypes that have only one gene (*Lr74*): Giza 171 (G2), Sakha 93 (G3), Gemmeiza 7 (G6), Gemmeiza 10 (G8), Sids 14 (G15), G19, G24, G26, G27 and G33. Genotypes in this group recorded low AUDPC values except for G2, G3, G6, G8, and G15, which recorded AUDPC of more than 300 and susceptible reactions as the final disease severity (Tables 5 and 6). The third group includes genotypes containing two genes, *Lr34* and *Lr74* (Sakha 94, G4, Sids 13, G14), while G43 contains the *Lr34* and *Lr74* genes and Beni-Suef 6 (G18). G20, G22, and G29 have

Lr75 and *Lr80* genes, while G10 (Misr 1) and G13 carry *Lr74* and *Lr75* genes; Gemmeiza 9 (G7), G21, and G35; carry *Lr74* and *Lr80* genes; and G47 has *Lr74* and *Lr75* genes, while G21 carries *Lr74* and *Lr80* genes. All genotypes in this group recorded low AUDPC values except genotypes Gemmeiza 9 (G7) and (Beni-Suef 6) G18, which have high AUDPC values (Table 6). The fourth group, including genotypes, contains three genes, Under this group, there are 21 genotypes with different combinations, described as follows: Sakha 95 (G5), Shandaweel 1 (G16), and G45 carry *Lr34*, *Lr74*, and *Lr80* genes, while Giza 168 (G1), G9, Sids 1 (G12), Beni-Suef 5 (G17), G25, G28, G30, G31, G32, G36, G37, G38, G39, G41, G42, and G46 carry *Lr74*, *Lr75*, and *Lr80* genes; G40, and G44 carry *Lr34*, *Lr74*, and *Lr75* genes. All genotypes recorded low AUDPC scores except genotype Sids 1 (G12) has a high AUDPC value (Table 6). The fifth group includes the genotypes containing four genes, and all these genotypes recorded low AUDPC scores (Table 6). G34 carries *Lr34*, *Lr74*, *Lr75*, and *Lr80* genes, while Misr 3 (G11) carries *Lr34*, *Lr74*, *Lr75*, and *Lr80* genes.

DISCUSSION

Leaf rust, caused by *Puccinia triticina*, causes noticeable losses in wheat production and reduces grain quality (Sayre et al., 1998). Studies confirmed that genetic resistance is the most effective method to fight disease infection (Vida et al., 2009) and environmentally safe (El-Orabey et al., 2014; Shahin and El-Orabey, 2016; El-Orabey et al., 2019 b). Genetic resistance is divided into two categories: 1) race non-specific resistance (named slow-rusting resistance, durable resistance, partial resistance (PR), or minor gene resistance) (Lowe et al., 2011). This type clarifies the ability of genotypes to slow down the progress of rust infection despite the infection type of the cultivated genotype (Caldwell, 1968), which is suitable for a broad spectrum of resistance to widespread races or new emergence races (Miedaner and Korzun, 2012). 2) race-specific resistance (named gene-for-gene resistance, or major gene resistance) (El-Orabey et al., 2019 a), which is associated with the rapid death of infected cells, and this phenomenon is called “hypersensitive response." (Ellis et al., 2014).

Therefore, the present study aimed to find new sources of durable resistance and new leaf rust resistance genes to overcome dramatic yield losses caused by disease infection. Using molecular markers, fifty genotypes were screened for slow rusting genes *Lr34*, *Lr74*, *Lr75*, and *Lr80*. Since *Lr34* was first characterized (Dyck, 1977, 1987), it has been proven that *Lr34* has remained durable for over fifty years (Krattinger et al., 2009; Lagudah et al., 2009). *Lr74* is located on chromosome arm 3BS in wheat. It was initially identified and mapped in the wheat varieties BT-Schomburgk and Spark (Kolmer et al., 2018 c). *Lr75* is an APR against leaf rust located on the short arm of chromosome 1B in wheat. It was initially detected in the Swiss cultivar Forno by Singla et al., (2017). *Lr80*, a recently discovered resistance gene, has been detected, and closely associated markers have been created. These markers can effectively combine *Lr80* with other genes that have been tagged with markers, enabling the creation of long-lasting control against leaf rust through gene pyramiding (McIntosh et al., 2017, 2020; Kumar et al., 2021).

Marker-assisted selection (MAS) was introduced in the progress of molecular breeding, which is superior to obvious phenotypic selection (Kumawat et al., 2020). In addition, MAS utilizes several rust resistance genes, and information about resistance genes estimated in different varieties can help breeders improve resistant varieties (Hanzalová et al., 2020). Therefore, molecular markers can be used beneficially to search for *Lr* resistance genes within the genetic resources to select parents for a successful breeding program (Atia et al., 2021). Gene pyramiding, accumulating many genes into one genotype, can provide more robust resistance (Nelson, 1978).

Four parameters: AUDPC, ACI, r-values, and RRI were used to evaluate the genotypes for leaf rust durability. These parameters were used as trusted estimators to determine the rust infection. Wang et al., (2005) and El-Orabey et al., (2019a) reported that the AUDPC is a powerful measure of adult plant resistance when evaluating plants under field conditions. Additionally, it was noted that genotypes that recorded low AUDPC values could indicate a high level of adult plant resistance (El-Orabey et al., 2019 a; Pandey et al., 1989; Lal Ahamed et al., 2004; Singh et al., 2005; Boulot, 2007).

Generally, almost half of the genotypes recorded a decline in resistance in the second season due to high disease pressure and the appearance of new races. The decline in resistance was based on the genotype's genetic background, including the genetic content of slow-rusting genes. These results agreed with the variance analysis, showing the significant effect of genotypes, environment, and genotype-environment interaction on the differences among genotypes regarding leaf rust infection. That is fitting with Singla et al., (2017), whose research explained clear evidence about the effect of different resistance combinations do not act in the same way in all environments.

Tested wheat genotypes were evaluated under field conditions against leaf rust disease for two growing seasons (2019/20 and 2020/21) at the adult plant stage. Based on AUDPC, ACI, and r-values (Table 6), the evaluation results revealed that most of the tested genotypes exhibited high to moderate leaf rust resistance, showing a high level of partial resistance, except Sakha 93, Gemmeiza 7, Gemmeiza 9, and Sids 1, which showed the highest values of all parameters and were classified as fast rusting genotypes, These results were shown by using cluster analysis based on AUDPC (Figure 1). These results agree with the results obtained by El-Orabey et al., (2019a). In addition, Fahmi et al., (2015) confirmed that the Sids 1 variety was considered a fast-rusting variety, which agreed with the present results.

All the previous fast-rusting genotypes have the *Lr74* gene. In addition, Gemmeiza 7 contains the *Lr67* gene, Gemmeiza 9 has the *Lr46* gene, and Sids 1 has the *Lr75* gene (Table 7). Although these genes did not confer these genotypes' partial resistance, they may need more slow rusting genes introduced to accumulate their effects and reveal a high level of durable resistance. This suggestion matches the study of Huerta-Espino et al., (2020), which demonstrated that the level of slow rusting resistance depends on the number of slow rusting resistance alleles that already exist in the cultivars, as well as it was previously noticed by the cultivar. Additionally, Singh et al., (2000) demonstrated the need for two to three slow rusting genes in the genotype to achieve a near-immune response to leaf rust.

It was noticed that there are some partially resistant genotypes, such as Beni-Suef 5, and fast rusting genotypes, such as those containing the same slow rusting genes, *Lr75*, *Lr74*, and *Lr80* (Table 7). This result can be attributed to the genetic background and the existence of another new slow-rusting gene. These results prove the minor effect of the slow-rusting genes *Lr74* and *Lr75*. QLr.hwwg-3BS1 provisionally identified as *Lr74*, which has been studied in the Clark population by Li et al., (2017). Their results confirmed the significant effect of *Lr74* QLr.hwwg-3BS.1, which accounts for 12-13% of the reaction in leaf rust severity and performs better in combination with other genes (Li et al., 2017). Additionally, Singla et al., (2017) proved that *Lr75* has been shown to provide an additive effect when acumelated with another slow rusting. Furthermore, Herrera-Foessel et al., (2011) reported that the optimal combination of resistance genes that produce the perfect resistance effect is still generally unclear.

On the other hand, Sakha 94, Sakha 95, Sids 12, BW55751, BW50949, BW55230, BW56961, BW55161, and BW55243 genotypes revealed MR-R infection type (Table 5) and very low ACI (Table 6) in both growing seasons, showing complete resistance resulting from major gene effects. At the same time, these genotypes revealed durable resistance. These findings in the present study align with prior research by El-Orabey et al., (2019a), indicating that Giza 171 and Misr 3 exhibited infection-type or moderate resistance. These cultivars possess partial resistance genes, and they mentioned the possibility that the resistance trait results from one or more major gene expressions.

The *Lr80* gene is a new leaf rust resistance gene used in pyramiding to achieve durable resistance (Kumar et al., 2021). This gene was detected in twenty-six out of fifty tested genotypes (Figure 3D and Table 7), including fast-rusting and partial-resistant genotypes. This suggests that the *Lr80* gene may be minor, as its effect is based on the genotype's genetic background. Available information about this gene is still limited.

Three lines, BW56948, BW56949, and ACSAD#14, displayed high FRS percentage and AUDPC values (Tables 5 and 6), showing partial resistance, although they did not possess any of the tested slow rusting genes. The resistance in these genotypes may have resulted from the existence of some minor genes that have not been tested in the present genotypes or have not been discovered until now (Imbaby et al., 2014; Pinto da Silva et al., 2018).

The genotypes containing the *Lr34* gene, such as Sakha 94, Sakha 95, Sids 13, Shandaweel 1, BW55208, and BW55193 (Table 7), revealed high partial resistance (Table 6) regardless of genetic background. The *Lr34* gene has a major effect on leaf rust resistance. *Lr34* is not considered race specific. It ensures the general resistance of adult plants to leaf rust and resistance to various pathogen pathotypes (Singh and Rajaram, 1992). In this respect, the gene is very valuable. Its presence increases the general resistance of wheat cultivars.

All genotypes containing three or more of the tested slow rusting genes, including the *Lr80* gene, revealed high partial resistance, such as Misr 1, Misr 2, Misr 3, BW55751, and BW55730, as shown by Figure 2, which showed a negative correlation between the number of slow rusting genes and the values of the partial resistance parameters. The highest number of slow rusting genes was observed in Giza 168, Misr 3, and BW55213 (Table 7). Herrera-Foessel et al., (2012) reported that the wheat genotype carries only one of two resistance genes, *Lr68* or *Lr34*, in their genetic background and records less resistance than the genotype that carries both genes. In Mexico, Lillemo et al., (2011) mentioned supported results, showing that the effect of *Lr68* was less than that of *Lr34* and *Lr46*. The slow rusting genes *Lr34*, *Lr46*, *Lr67*, and *Lr68* can be considered backbone genes and, when present in combination with other major genes and with known or unknown small effect or minor genes (QTLs), have provided effective resistance over the years in wheat improvement (Ellis et al., 2014).

Sids 14 has only the *Lr74* gene; Beni-Suef 5 has the *Lr74*, *Lr75*, and *Lr80* genes; and Beni-Suef 6 has the *Lr75* and *Lr80* genes (Table 7). They show partial resistant genotypes that display low FRS percentage (Table 5) and AUDPC (Table 6) values, except for Sids 14, which revealed fast leaf rusting, attributing to the existence of one tested minor gene. A previous search reported that the same previous genotypes did not possess any of the four genes: *Lr34*, *Lr46*, *Lr67*, and *Lr68*. The resistance in these varieties may have resulted from the existence of some minor genes or one of the newly characterized slow rusting resistance genes, *Lr75*, *Lr77*, and *Lr78* (Pinto da Silva et al., 2018; Imbaby et al., 2014). These previous results supported the present results.

Wheat genotypes that displayed MS infection type may possess slow rusting resistance. Disease development progressed gradually and was highly overdue within cultivars. Such partially resistant lines could delay the evolution of new virulent races of the pathogen because multiple-point mutations are extremely rare in normal situations (Schafer and Roelfs, 1985; Ali et al., 2008; Tsilo et al., 2010). The same matching result was reported by Narute et al., (2005) and Draz et al., (2015).

Research focusing on the genetic basis of rust disease and developing wheat varieties resistant to leaf rust has been crucial (Kumar et al., 2022). It is generally viewed as ideal to have both ASR (all-stage resistance) and APR (adult plant resistance) genes expressed together in the same cultivar to achieve effective leaf rust resistance (Pinto da Silva et al., 2018). As a possible consequence, this result may be considered a powerful source of information about resistance genes for the tested genotypes, and we need to establish more studies about the suitable combination of APR genes with effective race-specific genes in our local varieties to magnify the genotype performance to leaf rust resistance under field conditions.

CONCLUSION

The present study yielded positive outcomes, as certain wheat genotypes that displayed resistance or moderate resistance against *Puccinia triticina* were identified. These genotypes, especially the genotypes that have the highest number of slow rusting genes, could serve as valuable sources of genetic material for managing the disease in national programs and building up new effective breeding programs. Consequently, we recommended using these genotypes in pyramiding for durable resistance in breeding programs.i.e (Giza 168, Misr 3, and BW55213).

This study evaluated 50 Egyptian wheat genotypes for their leaf rust resistance level at the adult plant stage for two successive seasons. The present study provides valuable information about the genetic characterization of *Lr74*, *Lr75*, and *Lr80* in fifty tested genotypes as a powerful source of resistance in breeding programs. Our results demonstrated that the *Lr74* gene was the most frequent, detected in 86% of the tested genotypes. In contrast, the *Lr67* gene had the lowest frequency detected in 14% of the genotypes.

Furthermore, we need more investigations to explore the genetic background of the tested genotypes and to focus more on studies that provide information about perfect resistance gene combinations.

DISCLOSURE STATEMENT

The authors report that there are no competing interests to declare.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are all included in the manuscript.

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