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Phenotyping and validation of molecular markers associated with rust resistance genes in wheat cultivars in Egypt

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Abstract
Background Thirteen Egyptian wheat cultivars were evaluated and characterized for adult plant resistance to yellow, leaf, and stem rusts. SSR markers linked to yellow, leaf and stem rust resistance genes were validated and subsequently used to identify wheat cultivars containing more than one rust resistance gene.

Results Results of the molecular marker detection indicated that several genes, either alone or in different combinations, were present among the wheat cultivars, including Yr, Yr78 (stripe rust), Lr, Lr70 (leaf rust), Sr, Sr33, SrTA10187, Sr13, and Sr35 (stem rust), and Lr34/Yr18 and Lr49/Yr29 (leaf/stripe rust). The cultivar Sakha-95 was resistant to leaf and stem rusts, and partially resistant to stripe rust; however, this cultivar contained additional rust resistance genes (Lr, Sr and Lr/Yr). The area under the disease progress curve (AUDPC) type for the various wheat cultivars differed depending on the type of rust infection (yellow, leaf, or stem rust, indicated by Yr, Lr, and Sr). The cultivars Gem-12, Sids-14, Giza-171, and Giza-168 had AUDPC types of partial resistance and resistance. All six cultivars, however, contained additional rust resistance genes.

Conclusions Marker-assisted selection can be applied to improve wheat cultivars with efficient gene combinations that would directly support the development of durable resistance in Egypt. Once the expression of the resistance genes targeted in this study have been confirmed by phenotypic screening, the preferable cultivars can be used as donors by Egyptian wheat breeders. The results of this study will help breeders determine the extent of resistance under field conditions when breeding for rust resistance in bread wheat.

Keywords Rust · Wheat cultivars · Resistance genes · Validation · SSR markers

Introduction
Wheat yellow rust, also known as yellow rust, is caused by Puccinia striiformis Westend f. sp. tritici. It occurs at high altitudes in temperate zones worldwide [1]. Yield losses from yellow rust can be considerable, ranging from 40% loss to complete destruction of the crop, depending upon the growth stage at which the disease attacks [2]. Planting a crop with diverse genetics is the most economical and environmentally safe method for controlling this disease.

Leaf rust, caused by Puccinia triticina, is one of the most common diseases of wheat, occurring nearly everywhere wheat is grown [3]. In Egypt, wheat cultivars lacking adequate resistance to leaf rust can suffer yield losses of 5–10% or more [4]. Wheat stem rust, caused by Puccinia graminis Pers. f. sp. tritici (Eriks. and E. Henn.), is still the biggest biotic threat to Egyptian wheat production. Wheat stem rust affects the entire wheat crop, especially during the late spring. Infection results in blockage of the vascular system, which leads to stunting and lodging of weak stalks, eventually causing severe yield losses as high as 100% due to shriveled grain and damaged tillers [5]. In Egypt, yield losses from stem rust ranged from 1.96 to 8.21% on Egyptian wheat cultivars [6].

Recurring wheat rust diseases cause considerable yield losses worldwide. To prevent yield loss, different fungicides are used, either alone or in combination, to respond to increased disease aggressiveness under field conditions. During the growing seasons of 2018/2019 and 2019/2020...
in Egypt, the spread of yellow rust in wheat led to the consumption of many fungicides to combat widespread crop disease [7]. Adult plant resistance (APR) has often been considered a type of polygenic resistance [8]. This form of resistance protects wheat cultivars against yellow, leaf, and stem rust races by pyramiding many resistance genes in a single variety, thereby conferring a high level of generalized resistance against the target pathogen race. In this respect [9], stated that breeding programs should develop and release rust resistant cultivars, conditioning them with both race-specific and race-nonspecific resistance genes. The identification of genes conferring APR to wheat stem rust would be an initial and significant step towards effectively controlling this disease.

The best approach in preventing yield loss from wheat rusts is to follow a durable disease resistance program in commercially adopted cultivars that have otherwise good agronomic traits, but are susceptible to disease. Using resistant cultivars is the cheapest, most reliable, and most environmentally friendly way to control rust disease. The primary focus of any disease resistance breeding program is to work on achieving durable resistance, which often involves identifying the race-nonspecific or slow-rusting yellow, leaf, and stem rust resistance genes with molecular markers [10].

Marker-assisted selection (MAS) has been broadly used; however, breeding methods for MAS depend on both phenotypic and genotypic selection. In wheat, MAS may be achieved using a robust DNA molecular marker firmly associated with the resistance genes Lr, Yr, and Sr [11]. Closely linked markers give phenotype-unbiased choices of the linked genes in the cultivars. Such molecular markers confirm the identification of marked genes with close genetic similarity to the cultivar in question.

Yellow rust, a destructive disease of wheat, causes significant yield loss [12]. Validation and characterization of wheat genotypes for the yellow rust resistance gene Yr78 has been attempted using DNA bulked segregant analysis (BSA), resistance gene analog polymorphism (RGAP), and simple sequence repeat (SSR) techniques [12]. Molecular markers linked to the resistance gene Yrwhh2 have been identified, making these markers potentially useful for improving yellow rust resistance in wheat cultivars when used in integration with other genes [13]. The validation of a polymorphic fragment linked to Yr10 was tested using the marker RAPD OPE5. The resulting 1100 bp fragment was found in all resistant BC4F5 lines, and was absent in all susceptible lines tested [14].

The markers gwm389 and BS0062676 flanked Yr57 and were genotyped on a set of Indian and Australian wheat cultivars. Cultivars known to lack Yr57 showed an absence of resistance-linked alleles from these markers. These markers would be useful in marker-assisted pyramiding of Yr57 with other marker-tagged major and minor genes [15]. Haplotype analysis identified specific SNPs linked to Yr26 and advanced robust and breeder-friendly KASP markers. This integration strategy can be applied to speed-generate many markers that are closely linked to target genes [16]. The development, validation, and re-selection of wheat genotypes with the pyramided genes Yr64 and Yr15 are linked to increased yellow rust resistance. These genotypes, with two effectively high genes, should be more helpful than individual gene genotypes in the development of high-level, durably resistant wheat genotypes [17]. The SSR markers Xgwm533, wmc580, cfa2123, and barc71, which are linked to the stem rust resistance genes Sr2, Sr13, Sr22, and Sr24, are useful in the MAS of stem rust resistance genes in Egypt [18]. The molecular markers barc8 and gwm11, linked to Yr15, were used for foreground selection and selection of the advanced genotypes WBM3682 and WBM3684 [19].

The SSR markers barc71 and xucw108 were linked to the rust resistance genes Lr24/Sr24 and Lr37/Sr38/Yr17, respectively [20]. In backcrossed plants, rust resistance was transferred from FLW20, and the SCAR marker SCS265512 was used to validate the outcomes of Lr19 in a host–pathogen interaction (HPI) test. Molecular marker-assisted validation for Lr19 showed 88–93% consistency, indicating that both of these techniques must be mutually exclusive for accurate and effective selection of Lr19 [21, 22] examined five SNP markers linked to Lr48 (IW31002, IW39832, IW34324, IW72894, and IW36920) and KASP markers on wheat lines. The SCAR marker SCS1302 for Lr24/Sr24 was used to select plants carrying the respective gene(s). The findings of this investigation proved the usefulness and importance of MAS in precise introgression of genes conferring leaf rust resistance. The validation of the leaf rust resistance gene LrLC10 (Lr13) and its co-segregation markers in wheat genotypes was reported by [23].

In the last few years, new wheat rust races (warrior races) have been found to be more aggressive and tolerant of high temperatures than previously seen. In Egypt, the appearance of new yellow rust races resulted in lost resistance in several of the most resistant cultivars, such as Gemmeiza 11 and Sids 12, and most other Egyptian wheat cultivars. Moreover, the lack of genetic diversity among Egyptian wheat cultivars is a serious problem that could increase the virulence of yellow rust, potentially causing a huge reduction in Egyptian wheat production [24, 25]. Therefore, the aims of this study were (1) to more accurately evaluate and characterize the APR of thirteen Egyptian bread wheat cultivars to yellow, leaf, and stem rust under both artificial inoculation conditions and natural infection conditions in the field; (2) to identify effective genes for controlling yellow, leaf, and stem rust diseases in the tested wheat cultivars using SSR markers; and (3) to identify wheat cultivars containing more than one rust resistance gene.
Materials and methods

Thirteen cultivars of spring wheat cultivated in Egypt have been used, and they are described in (Table 1). These cultivars were obtained from Wheat Research Section, Field Crops Research Institute (FCRI), Agricultural Research Center (ARC), Ministry of Agriculture, Egypt. Furthermore, any field activities were conducted properly within the Egyptian laws and regulations by an Agriculture research center (ARC) specialist (Second author on this paper). Therefore, no specific permissions were required for locations or field activities. Furthermore, we confirm that the field studies conducted in the current study did not involve endangering indigenous or protected species. Each cultivar was planted in 2 m long rows with four replicates using a randomized complete block design (RCBD). Recommended agricultural wheat practices were applied. The plots were surrounded by a spreader area planted with a mixture of highly susceptible wheat varieties, including *Triticum spelta sahariensis*, Morocco, Thatcher, and Max, to spread stem rust inoculum and increase the disease pressure. For field inoculation with yellow, leaf, and stem rust, the spreader plants were misted with water and then dusted with a mixture of uredinio spores of the most prevalent rust races, mixed with talcum powder at a rate of 1 (spores): 20 (talcum powder). All wheat plants were inoculated at the booting stage, according to the method of [26].

Disease assessment

Disease assessment was performed over two seasons of the study when the susceptible wheat varieties expressed 50% rust severity. The percentage rust severity was recorded separately for yellow, leaf, and stem rusts based on a modified Cobb’s scale of 0–100% [27]. The host response assessment also included recording the infection type (IT), according to [28]: Tr = trace, R = resistant, MR = moderately resistant, R-MR = resistant to moderately resistant, MR-MS (also abbreviated as M) = moderately resistant to moderately susceptible, MS-S = moderately susceptible to susceptible, MS = moderately susceptible, and S = susceptible. The final disease severity score was obtained for each individual by multiplying the individual’s IT assessment by its numerical value, where Tr = 0.1; R = 0.2; MR = 0.4; M = 0.6; MS = 0.8; and S = 1.0; each genotype’s scores were then averaged to give the average coefficient of infection (ACI) [28]. Disease severity scores were used to estimate the area under the disease progress curve (AUDPC), which was calculated for each genotype according to an equation proposed by [29], as follows:

\[
\text{AUDPC} = \frac{D}{2} \left( \frac{Y_1 + Y_K}{2} + \sum_{2}^{K-1} Y_i \right)
\]

where \(D\) = Time interval (days between consecutive records); \(Y_1 + Y_K\) = Sum of the first and final disease scores; \(Y_2 + Y_3 + \ldots + Y_{(K-1)}\) = Sum of all in-between disease scores.

Table 1 Name, pedigree, and year of release of thirteen wheat genotypes used in this study

| Genotypes   | Pedigree                                                                 | Year of release |
|-------------|---------------------------------------------------------------------------|-----------------|
| Gemmeiza 9 (Gm-9) | Ald“S”/Huas//CMH74A.630/SxCGM4583-5GM-1GM-0GM                          | 1999           |
| Gemmeiza 10 (Gm-10) | MAYA74“S”/0 N/160–147/3/BB/GLL4/“CHAT=S”/S/CROW“S”. GM5820-3GM-1GM-2GM-0GM | 2004           |
| Gemmeiza 11 (Gm-11) | BOW“S”/KVZ“S”//7C/SER182/3/GIZA168/SAKHA61GM5820-3GM-1GM-2GM-0GM | 2011           |
| Gemmeiza 12 (Gm-12) | OTUS/3/SARA/THB//VEECCMS97Y00227S-5Y-010 M-010Y-010 M-2Y-1 M-0Y-0GM | 2011           |
| Sids-1       | HD2172/Pavon “S”//1158.57/Maya74 “S” SD46-4Sd-2SD-1SD-0SD                | 1996           |
| Sids-12      | BUC//7C/ALD//MAYA74/ON/1160.147/3/BB/GLL4/“CHAT=S”/6/MAYA/VUL//CMH74A.630//4SXSD7096-4SD-1SD-1SD-0SD | 2007           |
| Sids-13      | AMAZ19 = KAUZ“S”//TSI/SNB“S”//ICW94-0375-4AP-2AP-03AP-0AP-3AP-0AP-0AP-0AP | 2010           |
| Sids-14      | KAUZ“S”//TSI/SNB“S”. ICW94-0375-4AP-2AP-03AP-0AP-3AP-0AP-0AP-0AP-0AP | 2014           |
| Giza-168     | MRL//BUC/SERLCM93046-8 M-0Y-0 M-2Y-0B-0GZ                                | 1999           |
| Giza-171     | SAKHA 93/GEMMEIZA 9S.6-1GZ-4GZ-1GZ-2GZ-0S                                | 2013           |
| Misr-1       | OASIS/SKAUZ//4*BCN1312*PASTOR.CMSSOYO1881T-050 M-03Y-030 M-030WGY-33 M-0Y-0S | 2011           |
| Misr-2       | SKAUZ/BAV92. CMS96M03611S-1 M-010SY010M-010SY-8 M-0Y-0S                 | 2011           |
| Sakka-95     | POSTOR//SITE/MO/3/CHEN/AEGILOPS/SQARROSA(TAUS)                           | 2018           |
The rate of rust disease increase (r-value) was also estimated as a function of time, according to the formula by [30]:

\[ r\text{-value} = \frac{1}{t^2 - t_1} \left( \log e \frac{X_2}{1 - X_2} - \log e \frac{X_1}{1 - X_1} \right) \]

where \( X_1 \) = the proportion of susceptible infected tissue (disease severity) at date \( t_1 \); \( X_2 \) = the proportion of susceptible infected tissue (disease severity) at date \( t_2 \); \( t_2 - t_1 \) = the interval in days between the dates \( t_1 \) and \( t_2 \).

**Statistical analysis**

Combined analysis of the obtained data about rust diseases over the two seasons are presented in (Table 3), as outlined by [31]. Mean comparisons for variables were made among genotypes using least significant difference (LSD) tests at \( \alpha = 0.05 \). Homogeneity of the variance across two seasons was tested following the Bartlett’s Test [32].

**DNA extraction and SSR analysis**

Young leaves from each cultivar were removed and frozen (0.5 g; derived from the shoot tips), then ground to a powder in a mortar with liquid nitrogen. The genomic DNA of each cultivar was extracted using a Wizard Genomic DNA Purification Kit (PROMEGA Corporation Biotechnology, Madison, Wisconsin, USA). After extraction, the samples were treated with RNase and maintained at a temperature of \(-20°C\). The DNA quality was checked by electrophoresis on 0.8% agarose gel, and DNA concentration was determined using an Epoch multi-volume spectrophotometer (Thermo Scientific, USA). The quantified DNA stock was diluted to a final concentration of 25 ng \( \mu l^{-1} \). Twenty-one SSR markers linked to rust resistance genes in wheat were used (Table 2). Several studies have previously reported linkage of these microsatellite primers with rust resistance genes [12, 16, 32–43]. The polymerase chain reaction (PCR) mixture consisted of 20–50 ng of genomic DNA, 1 × PCR buffer, 1.5 mM MgCl₂, 0.1 mM dNTP, 0.5 μM primer, and 1 U Taq polymerase, in a volume of 0.025 cm³. The PCR program for SSR analysis consisted of an initial denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 50–61 °C (depending on the individual SSR primers) for 1 min, extension at 72 °C for 2 min, and final extension at 72 °C for 10 min. The amplification products were electrophoretically resolved on 3% (m/v) agarose gels containing 0.1 μg cm⁻³ ethidium bromide, and photographed on a UV trans-illuminator. The use of PCR is useful for the validation of resistance genes according to [15, 17, 21, 31].

**Data handling**

The SSR data was scored based on the presence or absence of amplified products for each primer, after excluding the unreplicable bands. Products found to be present in a given wheat cultivar were designated as “+” and products found to be absent were designated as “−”.

**Results**

The Bartlett’s Test results showed homogeneous variance across the two seasons for the studied traits. Therefore, a combined analysis of variance was performed for all studied traits.

The thirteen wheat cultivars differed significantly in their responses to yellow, leaf, and stem rust disease, as shown by the phenotypic expression of disease parameters (Table 3 and Supplementary Table S1).

The wheat cultivars Gem-12, Sids-14, Giza-171, and Sakha-95 displayed high APR to yellow rust, showing ITs of MR or MS. These cultivars also showed the lowest values of final rust severity (FRS), AUDPC, and r-value. Conversely, Giza-168 had a low IT, r-value, and AUDPC, which indicates that this cultivar had partial resistance to yellow rust. The remaining eight cultivars showed the lowest levels of field resistance to yellow rust infection; these had the highest recorded FRS, as well as relatively high AUDPC and high r-values (Table 3 and Supplementary Table S1).

The wheat cultivars Gem-12, Sids-14, Giza-171, Misr-1, and Sakha-95 had partial resistance to leaf rust (IT of MR to MS), AUDPC of less than 150, and the lowest r-values. The other wheat cultivars presented as susceptible to leaf rust symptoms, with reactions of 10 S to 80 S, AUDPC greater than 170, and the highest r-values (Table 3 and Supplementary Table S1).

Stem rust disease severity could be ranked into three main groups. The first group included the seven wheat cultivars Gem-12, Sids-1, Sakha-95, Gemmeiza-11, Sids-13, Giza-168, and Giza-171 (resistant cultivars), which exhibited the highest levels of resistance or partial resistance. This group had the lowest AUDPC estimates (less than 300.00), and were designated as partially resistant and slow-rusting cultivars. However, this group displayed the highest level of APR and field resistance to stem rust infection throughout the study, indicating that these cultivars may have durable resistance to stem rust. The second group included three wheat cultivars (Gem-9, Gem-10, and Sids-12), which showed intermediate stem rust resistance. These cultivars had FRS values of 20 S, 10 S, and 20 S, respectively, with intermediate AUDPC values and low r-values. This group had the lowest levels of APR.
to stem rust infection under field conditions. The third group included the wheat cultivars Misr-1 and Misr-2, which showed high FRS of 70 S and 60 S, respectively. These two cultivars had the highest AUDPC and highest r-values, and could therefore be classified as highly susceptible or fast-rusting cultivars (Table 3 and Supplementary Table S1).

**Validation of resistance genes (yellow, leaf, and stem rust) in wheat cultivars**

Simple sequence repeat molecular markers were amplified to validate the resistance genes *Yr*, *Sr*, and *Lr* in all thirteen Egyptian wheat cultivars (Table 4).
Validation of markers linked to yellow rust resistance genes

The SSR marker barc147-3B was linked to the Yr resistance gene. The marker’s bands showed amplification in the range of 115–150 bp. The 150 bp band was present only in Sids-12, which was a susceptible cultivar, whereas the 115 bp band was present in eight cultivars (Gem-9, Gemmeiza-10, Gem-11, Gem-12, Sids-1, Sids-14, Giza-171, and Misr-1). These cultivars had AUDPC types of partial resistance (PR: Gem-12, Sids-1, and Sids-14), resistance (R: Giza 171), and susceptible (Sus: Gem-9, Gem-10, and Gem-11) (Table 4; Fig. 1A). The SSR marker barc180-3B was linked with Yr78. Four genotypes (Gm-12, Sids-1, Sids-13, and Giza-168) showed the presence of Yr78 with a band size of 150 bp. Three of these cultivars had an AUDPC type of PR, whereas Sids 13 was of the type Sus. Nine cultivars (Gm-9, Gm-10, Gm-11, Sids-12, Sids-14, Giza-171, Misr-1, Misr-2, and Sakha-95) did not contain Yr78 (Table 4; Fig. 1B).

Validation of markers linked to leaf rust resistance genes

The marker barc64-7A amplified a 200 bp fragment for the leaf rust resistance gene. This marker was present in eight genotypes (Gm-9, Gm-10, Gm-11, Gm-12, Sids-13, Sids-14, Giza-171, and Misr-2); all eight of these cultivars were of the AUDPC type PR, except for Gm 9, which was AUDPC type Sus. Eight genotypes indicated the presence of the leaf rust resistance gene with a band size of 200 bp, whereas five genotypes did not contain this gene (Table 4; Fig. 1Aa). The SSR molecular marker barc130-5D exhibited linkage with the Lr70 leaf rust resistance gene present on chromosomal locus 5D. This marker showed amplified bands of 285 bp, which were present in all thirteen genotypes. Of these, ten were AUDPC type PR (Gm-10, Gm-11, Gm-12, Sids-12, Sids-13, Sids-14, Giza-168, Giza-171, Misr-1, and Misr-2); one AUDPC type R (Sakha-95); and two type Sus. (Gm-9 and Sids-1) (Table 4; Fig. 1Bb). The marker barc167-2B amplified a 255 bp fragment for leaf rust resistance. This marker was present in three genotypes (Gm-11, Sids-14, and Sakha-95), of which two were AUDPC type PR (Gm-11 and Sids-14), and one was type R (Sakha-95) (Table 4; Fig. 1Cc).

Validation of markers linked to stem rust resistance genes

The SSR marker barc104-6A was linked to the Sr13 gene. This marker had an amplified band size of 250 bp, which was present in all thirteen genotypes. Of these, five were AUDPC type PR (Gm-9, Gm-10, Gm-11, Giza-171, and Misr-2), of which five were AUDPC type PR (Gm-9, Gm-10, Gm-11, Giza-171, and Misr-2), and two were type R (Sids-13 and Sids-14). This resistance gene was absent in the genotypes

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**Table 3** Final rust severity (FRS), area under disease progress curve (AUDPC), and rate of rust disease increase (r-value) of yellow, leaf, and stem rust evaluated for thirteen Egyptian wheat cultivars grown under field conditions at Sids Research Station during the 2019/2020 growing season

| Genotypes | Yellow rust | Leaf rust | Stem rust |
|-----------|-------------|-----------|-----------|
|           | FRS | AUDPC | r-Value | FRS | AUDPC | r-Value | FRS | AUDPC | r-Value |
| Genotypes |      | Value | Type* |      | Value | Type |      | Value | Type |      | Value | Type |      | Value | Type |
| Gem-9     | 50 S | 423.50 | Sus   | 0.219 | 40 S | 315.00 | Sus | 0.200 | 20 S | 178.50 | P.R | 0.153 |
| Gem-10    | 50 S | 388.50 | Sus   | 0.219 | 30 S | 175.00 | P.R | 0.178 | 10 S | 80.50 | P.R | 0.114 |
| Gem-11    | 80 S | 577.50 | Sus   | 0.206 | 20 S | 175.00 | P.R | 0.153 | 5 MS | 28.00 | P.R | 0.053 |
| Gem-12    | 20 MS| 129.50 | P.R   | 0.147 | 20 MS| 84.00  | P.R | 0.140 | 0    | 0.00  | R   | 0.00  |
| Sids-1    | 30 S | 213.50 | P.R   | 0.178 | 80 S | 650.00 | Sus | 0.285 | Tr MR| 24.50 | R   | 0.033 |
| Sids-12   | 70 S | 700.00 | Sus   | 0.145 | 30 S | 175.00 | P.R | 0.178 | 18 S | 55.00 | P.R | 0.153 |
| Sids-13   | 60 S | 388.50 | Sus   | 0.238 | 30 S | 175.00 | P.R | 0.178 | TrMS | 28.00 | R   | 0.053 |
| Sids-14   | 20 MS| 122.50 | P.R   | 0.140 | 30 MS| 126.00 | P.R | 0.164 | TrMR | 24.50 | R   | 0.033 |
| Giza-168  | 10 S | 80.50  | P.R   | 0.114 | 20 S | 210.00 | P.R | 0.178 | 10 MS| 52.50 | P.R | 0.088 |
| Giza-171  | 10 MR| 52.50  | R     | 0.088 | 30 MS| 140.00 | P.R | 0.164 | 5 MS | 28.00 | P.R | 0.053 |
| Misr-1    | 40 S | 437.50 | Sus   | 0.121 | 20 MS| 112.00 | P.R | 0.140 | 70 S | 570.50 | Sus | 0.160 |
| Misr-2    | 50 S | 472.50 | Sus   | 0.140 | 10 S | 70.00  | P.R | 0.114 | 50 S | 353.50 | P.R | 0.219 |
| Sakha-95  | 10 MS| 59.50  | P.R   | 0.103 | Tr MR| 17.50  | R   | 0.053 | Tr MR| 28.00 | R   | 0.053 |
| Mean      | 378 | 346.35 | –     | 0.303 | –    | 134.14 | –   | 0.166 |      |       |     |       |      |      |
| LSD 0.05  | 1.79 |       | 1.85  | 1.67  |      |       |      |       |

*AUDPC type: Susceptible (Sus.) = AUDPC value greater than 300; Partial resistance (PR) = AUDPC value less than 300; Resistance (R) = AUDPC value less than 300 and infection type (IT) of 0, MR, Tr-MR, and Tr-MS. LSD 0.05 for AUDPC
Gm-12, Sids-1, Sids-12, Giza-168, Misr-1, and Sakha-95. The gene Sr13 is the only known gene to be operative against the TTKS complex of P. graminis f. sp. tritici; this includes the TTKSK (Ug99) race and its variants, TTKST and TTTSK (Table 4; Fig. 2A). The PCR-based diagnostic marker barc152-1B was linked to Sr33, which is found on chromosomal locus 1BS. All genotypes indicated the presence of this gene with a band size of 130 bp. Of these, seven were AUDPC type PR (Gm-9, Gm-10, Gm-11, Sids-12, Giza-168, Giza-171, and Misr-2); five were type R (Gm-12, Sids-1, Sids-13, Sids-14, and Sakha-95); and one was Sus. (Misr-1) (Table 4; Fig. 2B).

The marker barc173-6D was linked with the stem rust resistance gene SrTA10187, with a band size of 240 bp. This gene was found in ten cultivars (Gm-9, Gm-10, Gm-11, Gm-12, Sids-12, Sids-13, Sids-14, Giza-171, Misr-1, and Misr-2), of which seven were AUDPC type PR (Gm-9, Gm-10, Gm-11, Sids-12, Giza-171, Misr-1, and Misr-2) and three were type R (Gm-12, Sids-13, and Sids-14). This marker was absent in the remaining four cultivars (Sids-1, Giza-168, Giza-171, and Sakha-95) (Table 4; Fig. 2C). The marker barc200-2B was amplified as a 150 bp fragment for the stem rust resistance gene. This marker was present in two genotypes (Giza-171 and Sakha-95), which were AUDPC types PR and R, respectively (Table 4; Fig. 2E). The SSR marker wmc169 was linked with the stem rust resistance gene Sr35. This marker was amplified to a band size of 120 bp and was found to be present in seven cultivars (Sids-1, Sids-12, Sids-13, Sids-14, Gm-168, Misr-2, and Sakha-95), and absent in the remaining six cultivars (Table 4; Fig. 2D).

**Validation of markers linked to leaf/yellow rust resistance genes**

The SSR marker barc352-4D was linked with the leaf/yellow rust resistance gene Lr34/Yr18. Eight cultivars (Gm-10, Gm-12, Sids-1, Sids-12, Giza-168, Misr-2, and Sakha-95) indicated the presence of these genes with an amplified band size of 255 bp. Of these, four cultivars (Gm-12, Sids-14, Giza-168, and Sakha-95) were AUDPC types PR or R. The remaining five cultivars showed no introgression for these markers (Table 4; Fig. 3A). The SSR marker wmc44-1B, mapped on the long arm of chromosome 1B and linked to the leaf/yellow rust resistance gene Lr49/Yr29, was amplified to a band size of 242 bp. Out of the thirteen cultivars, six were positive for this marker (Gm-9, Gm-10, Gm-11, Sids-1, Sids-13, and Sids-14) and seven were negative (Table 4; Fig. 3B).

**Validation of markers linked to stem/yellow rust resistance genes**

The SSR marker wmc175-3A, mapped on the long arm of chromosome 3A and linked to the stem/yellow rust...
resistance genes \( Sr9 \) and \( Yr5 \), was used to identify the presence of \( Sr9 \) and \( Yr5 \) with an amplified band size of 260 bp. Out of the thirteen cultivars, seven (Gm-9, Gm-10, Sids-12, Sids-14, Giza-168, Giza-171, and Sakha-95) were positive for this marker. Of these, three cultivars (Sids-14, Giza-168, and Sakha-95) were AUDPC type PR, and one (Giza-171) was type R (Table 4; Fig. 3C).

**Identification of wheat cultivars containing more than one rust resistance dieses**

The results of molecular marker detection indicated that \( Yr \) (yellow rust); \( Lr \) (leaf rust); \( Sr \) (stem rust); and \( Lr/Yr \) (leaf/yellow rusts), were present alone or in different gene combinations among the wheat cultivars. The cultivar Sakha-95 was AUDPC type R for leaf and stem rusts, and PR for yellow rust dieses. However, Sakha-95 contained several other rust resistance genes (\( Lr \), \( Sr \) and \( Lr/Yr \)) (Table 4 and Supplementary Table S2). The AUDPC types for cultivars Gm-12, Sids-14, Giza-171, and Giza-168 were PR and R (Table 4 and Supplementary Table S2). The cultivar Sids-1 was recorded as PR, Sus, and R for \( Yr \), \( Lr \), and \( Sr \) respectively, whereas Sids-13 had respective AUDPC types of Sus, PR, and R for dieses (\( Yr \), \( Lr \), and \( Sr \)) respectively. Seven cultivars (Sakha-95, Gm-12, Sids-14, Giza-171, Giza-168, Sids-1, and Sids-13) contained more than one rust resistance gene (Table 4 and Supplementary Table S2). The phenotypic responses to infection by different rusts indicated the presence of additional slow-rusting resistance genes.

**Discussion**

The levels of field resistance (partial resistance) of wheat cultivars and their durability to yellow, leaf, and stem rust infections, were determined during this study using the three epidemiological parameters FRS, AUDPC, and \( r \)-value. The exploitation and deployment of this type of partial resistance comprise a major contribution to the genetic improvement of many crops, including wheat, in rust resistance breeding.
Fig. 2 Agarose gel electrophoresis showing allele sizes of the SSR markers A barc64, B barc130, C barc167, D barc200 and E wmc169 in 13 wheat cultivars.

Fig. 3 Agarose gel electrophoresis showing allele size of the SSR markers A barc352, B wmc44, and C wmc175 in thirteen wheat cultivars.
programs worldwide [7]. To increase wheat production in Egypt, breeding programs must select for both yield and disease resistance components, such as the traits studied in this investigation.

Rust diseases have a negative effect on wheat production, which can be attributed to the fact that the fungus causes extensive damage to the vascular system of the susceptible host plant, limiting the transportation of water and nutrients from the soil to the developing kernel and other organs. This in turn interferes with the translocation of photosynthates, which leads to shriveled grains [6]. Similar findings have been reported by numerous other research groups [44]. In highly susceptible varieties, the endosperm barely forms and the resultant grains are invariably completely shriveled.

The validation and characterization of APR for the yellow rust resistance gene Yr78 was explained by [12]. The SSR markers wmc737 and wmc494, and the SNP marker IWA7257, were used to test the presence of this gene. Expected PCR fragments of 871 and 537 bp were amplified from the positive control line T. turgidum ssp. The gene-based markers gwm455F3R, DArT-STS, and sun104 were genotyped on a set of thirteen Indian and 27 Australian wheat cultivars to screen the obscurity of alleles linked to the resistance gene Yr51, often referred to as negative validation. None of the genotypes tested were found to amplify the 225 bp allele linked to Yr51, indicating the fitness of this marker in MAS of the gene in these backgrounds. Therefore, sun104 can be used for MAS of Yr51 in wheat genotypes lacking the resistance-linked 225 bp allele [44]. The gene YrWh2 is flanked by the SSR markers wmc540-260 bp and Xgwm566-145 bp. Therefore, these two SSR markers can be used to ascertain the presence or absence of YrWh2 [13]. The gene Yr30 is linked with the SSR markers xgwm533 and xgwm493 [13]. The SSR marker gwm389-150 bp and SNP marker BS00062676 flank the YrAW2 and Yr57 genes for yellow rust resistance. Therefore, these two markers can be used to determine the presence or absence of YrAW2 and Yr57 [15]. The gene Yr60 confers moderate resistance to yellow rust in wheat. The marker wmc776 is linked with Yr60, and both of the SSR markers wmc313 and wmc219 were validated for this gene [43].

The SSR markers barc8 and xgwm493 are the nearest markers flanking Yr15. Fragments have an amplified band size of 221 bp with barc8, and 162 bp with Xgwm273 [45]. The yellow rust resistance gene YrJ22 is linked with the SSR marker wmc658 and the SNP marker IWA1348. These flanking markers could successfully identify resistant and susceptible alleles in wheat cultivars, and can be used for selecting YrJ22 in breeding programs [46]. The SNP markers CM1461, CM501, and WRS467 clearly distinguish wheat cultivars that harbor the genes Yr26, Yr24, YrCH42, and YrGn22, indicating that these markers could be used to confirm the presence of Yr26. Moreover, the combination of CM1461, CM501, and WRS467 appears to be the most predictive of Yr26, based on varietal panels [54, 55, 56, 57, 18]. The yellow rust resistance genes Yr64 and Yr15 are linked with the SSR markers barc8, Xgwm413, and Xgwm273. The presence of fifty F5 lines selected from the cross of (susceptible line AvS) × (resistant line RIL-Yr64/Yr15) signifies the presence of Yr15. Similarly, the SSR marker xgwm413, with an allele band size of 102 bp, indicates the presence of Yr64 [17]. According to [19, 24], the marker xgwm11 amplified a Yr15-specific 215 bp fragment; the same band size was present in all of the genotypes tested, confirming the presence of Yr15. The presence of Yr15 was also validated in selected genotypes using another closely linked marker, barc8. This marker amplified a 221 bp fragment that was present in all of the genotypes [19].

The leaf rust resistance gene Lr70, which has been newly mapped in the common wheat accession KU3198 [34], is linked with the SSR marker barc130. The SSR marker cfd20 is linked with the leaf rust resistance gene Lrk1 [34]. One hundred and sixty-one plants of the backcross (HS240 susceptible parent/FLW20 Lr19) were determined to be resistant following a HPI check; these were validated using the SCAR marker GSC265512, which is linked to Lr19. Of the original 161 plants, 150 were determined to be positive for Lr19 [21]. Molecular APR markers for the leaf rust resistance gene Lr48 were used in wheat by [47]. Five SNP markers (IWB31002, IWB39832, IWB34324, IWB72894, and IWB36920) were co-segregated with Lr48. The SSR markers sun563 and sun497 were linked with the leaf rust resistance genes Lr48 and Lr13, and the SSR markers Xgwm429 and barc7 were linked with Lr48 [47, 48] identified leaf rust resistance genes in wheat cultivars produced in Kazakhstan. They reported that the predictable marker pTAG621 fragment associated with Lr1 was detected in twelve out of 22 wheat cultivars tested. The markers F1.2245 and Lr10-6/f2, linked to Lr10, were found in only two wheat cultivars. The marker Gb-F and -R fragments specific to Lr19 were detected only in the cultivar Pallada from Russia [48]. The SSR markers Xgwm512 and cfd36 were found to be putatively associated with the leaf rust resistance gene LrM. The marker Xgwm512 conducted as a dominant marker and amplified an allele of 200 bp in the rust-resistant genotype Ae. markgrafii, whereas cfd36 behaved as a codominant marker and amplified an allele of 124 bp in the rust-resistant genotypes Ae. markgrafii and IL ER9-700. In the susceptible parent AL, cfd36 amplified two alleles of 110 and 192 bp, respectively [49]. The SSR marker wmc221 and GB markers were linked with the leaf rust resistance gene Lr19. These markers were used to select 25 wheat cultivars that were evaluated for leaf rust resistance under natural field infection conditions. The SSR marker wmc221 amplified a product of 200 bp, suggesting that the Lr19 gene was in only two of the 25 wheat cultivars tested. A band of 220 bp was
found in the remaining genotypes, indicating the absence of *Lr19* [50]. The markers CAUT163 and Lseq22 were linked with the leaf rust resistance gene *LrLC10*. Thirty-two wheat genotypes were identified by these two markers from the 984 F₂ homozygous susceptible plants, and were further genotyped with ten additional markers [23].

The resistance gene *Sr33* is flanked by the SSR markers barc152 and cfd15, whereas the gene *Sr45* is flanked by the SSR markers cfd21 and barc229. As a result, these SSR markers may be used to validate the presence of *Sr33* and *Sr45* [36]. The resistance gene *SrTA10171* in the validated population BC2F₁ was identified by the SSR markers wmc827 and barc173 as being polymorphic among resistant and susceptible genotypes. For the SSR marker wmc827, the donor parent, TA10171, had a 132 bp allele, and the susceptible recurrent parent, KS05HW14, had a 146 bp allele. For the SSR marker barc137, the donor parent, TA10171, had a 275 bp allele, and the susceptible recurrent parent, KS05HW14, had a 237 bp allele [38]. The development and validation of molecular markers linked with the stem rust resistance gene *Sr13* in durum wheat was described by [34]. The markers dupw167 and AFSr13 were validated on 21 durum wheat cultivars by incorporating smooth MAS of *Sr13* in segregating populations. Only the SSR marker gwm427 showed polymorphism, recognizing the presence of *Sr13* in ten of the fifteen backcross derivatives carrying *Sr13* from their *Sr13*-lacking recurrent parents [34]. The validation of markers linked to the stem rust resistance gene *Sr28*, which is effective against the race Ug99, was described by [51]. In [41], the SSR markers wmc332 and DART wPt-7004 were identified as linked to *Sr28* based on the amplification of different sized alleles from the resistant and susceptible genotypes. The marker wmc332 amplified alleles of 214 bp from the resistant genotypes and 208 bp or less from the susceptible genotypes, whereas the marker wPt-7004-PCR resulted in two amplicons of sizes 166 and 194 bp, respectively. Preferential amplification of the 194 bp amplicon was linked with the presence of *Sr28* [51], [39, 52] identified SSR markers of the stem rust resistance gene *Sr42* for efficient use in MAS and stacking of resistance genes in wheat breeding populations. The SSR marker cfd49 was linked to *Sr42*, producing an amplified fragment of 202 bp in resistant genotypes [39]. The SSR markers cfd49 and barc183 were found to flank a gene that was assumed to be *Sr42* in wheat genotypes [52]. A detected recombination between *Fhbl* and *Sr2* using molecular markers was reported by [40, 53, 54]. In these studies, UMN10 was a codominant marker (237 and 240 bp), whereas cssSr2 was a dominant marker (172 bp) for the wheat genotypes. A closely linked and codominant SSR marker, Xgwm533 (120 bp), was used to track *Sr2* in wheat genotypes [53]. Markers flanking csLV34–Xgwm295 were linked with the *Yr18/Lr34* genes, which confer effectively durable resistance to rust diseases [55, 56] and trace the origins of their rust resistance region to many current wheat cultivars. Using a diagnostic STS marker revealed that *Lr34/Yr18* is a significantly slow-rusting gene, conferring high levels of resistance when concerted with other minor genes [57], [58] identified close linkage of the SSR marker sun180 to the gene *Yr47/Lr52*. The amplification of a different sun180 amplicon (195 bp) than that linked with *Yr4/Lr52* (200 bp) in wheat genotypes explains its robustness for MAS of these genes. Among 34 F₂ wheat lines, 28 were positive for the SSR marker wmc221, indicating the presence of *Lr19/Sr25*. Out of fourteen chosen F₂ lines from F₂, nine were positive for *Lr19/Sr25*. Tightly linked SSR, STS, and AFLP markers were useful in the planning of the *Sr45/Lr21* locus. Sequences from an AFLP marker amplified a fragment that was linked with *Sr45/Lr21*. The STS marker cssu45 provided amplified fragments of 220 and 238 bp in the resistant and susceptible plants, respectively [34], [22] consolidated the rust resistance genes *Lr19/Sr25* and *Lr24/Sr24* in wheat through marker-assisted backcross breeding. Amplification using the marker xwmc221 produced the desired allele size of 200 bp, indicating the presence of *Lr19/Sr25* in the resistant genotypes, whereas a band of 220 bp indicated the absence of *Lr19/Sr25* in the susceptible genotypes. In the case of the marker SCS1302, a band of 609 bp, indicating the presence of *Lr24/Sr24*, was obtained in the resistant genotypes, whereas no band occurred in the susceptible genotypes [22].

**Conclusion**

The newly evolved wheat cultivars Gem-12, Sids-14, Giza-171, and Sakha-95 exhibited improved genetic resistance traits against yellow, leaf, and stem wheat rust diseases, as indicated by the lowest FRS, AUDPC, and r-values (Tables 3 and 4). Moreover, these cultivars contained multiple rust resistance genes. The phenotypic responses to different rust infections indicated the presence of additional slow-rusting resistance genes. Marker-assisted selection can be applied to improve wheat cultivars with efficient gene combinations that would directly support the development of durable resistance in Egypt. Once the expression of the resistance genes targeted in this study have been confirmed by phenotypic screening, the preferable cultivars can be used as donors by Egyptian wheat breeders. The results of this study will help breeders determine the extent of resistance under field conditions when breeding for rust resistance in bread wheat.

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Author contributions AE, extracted DNA and applied PCR reaction and analyses the markers and wrote the paper FBF, extracted DNA and applied PCR reaction and analyses the markers and wrote the paper WE, were responsible for evaluation of rust disease and wrote the section (rust disease). MA were responsible for evaluation of rust disease and wrote the section (rust disease). RME revised the paper and manage the whole work.

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Data availability Available.

Code availability No code.

Declarations

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval and consent to participate This article does not contain any studies with human participants or animals performed by any of the authors. The authors declare that the work is ethically approved and consented to participate.

Consent for publication The authors declare that the work has been consented for publication.

Permissions information Any field activities were conducted properly within the Egyptian laws and regulations by an Agriculture research center (ARC) specialist (Second author on this paper). Therefore, no specific permissions were required for locations or field activities. Furthermore, we confirm that the field studies conducted in the current study did not involve endangering indigenous or protected species.

Regulation The manuscript complies the local and national regulations.

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