Phenotyping and Validation of Molecular Markers Associated With Rust Resistance Genes in Wheat Cultivars in Egypt

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Research Article

Keywords: rust, wheat cultivars, resistance genes, validation, SSR markers

Posted Date: October 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-976250/v1

Version of Record: A version of this preprint was published at Molecular Biology Reports on November 29th, 2021. See the published version at https://doi.org/10.1007/s11033-021-07002-8.

Abstract

Thirteen Egyptian wheat cultivars were evaluated and characterized for adult plant resistance (APR) to yellow, leaf, and stem rusts. 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 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 (PR) and resistance (R). All six cultivars, however, contained additional rust resistance genes.

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. & 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 1.96–8.21% 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, 13, 14]. 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 [14]. Molecular markers linked to the resistance gene *Yrwh2* have been identified, making these markers potentially useful for improving yellow rust resistance in wheat cultivars when used in integration with other genes [15]. 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 fourteen resistant BC4F5 lines, and was absent in all susceptible lines tested [16]. The markers gwm389 and BS00062676 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 [17]. 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 [18]. 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 [19]. 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 [20]. The molecular markers barc8 and gwm11, linked to *Yr15*, were used for foreground selection and selection of the advanced genotypes WBM3682 and WBM3684 [21].

The SSR markers barc71 and xucw108 were linked to the rust resistance genes *Lr24/Sr24* and *Lr37/Sr38/Yr17*, respectively [22]. 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* [23]. [24] examined five SNP markers linked to *Lr48* (IWB31002, IWB39832, IWB34324, IWB72894, and IWB36920) 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 [25].

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 [26, 27]. 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 2m 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 [28].

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"/0N//160-147/3/BB/GLL/4/CHAT"S"/5/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//VEECMSS97Y00227S-5Y-010M-010Y-010M-2Y-1M-0Y-0GM	2011
Sids-1	HD2172/Pavon "S"//1158.57/Maya74 "S" SD46-4Sd-2SD-1SD-0SD	1996
Sids-12	BUC//7C/ALD/5/MAYA74/ON//1160.147/3/BB/GLL/4/CHAT"S"/6/MAYA/VUL//CMH74A.630/4*SXSD7096-4SD-1SD-1SD-0SD	2007
Sids-13	AMAZ19=KAUZ"S"//TSI/SNB"S". ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP-0SD.	2010
Sids-14	KAUZ"S"//TSI/SNB"S". ICW94-0375-4AP-2AP-030AP-0APS-3AP.	2014
Giza-168	MRL/BUC//SERI.CM93046-8M-0Y-0M-2Y-0B-0GZ.	1999
Giza-171	SAKHA 93 / GEMMEIZA 9S.6-1GZ-4GZ-1GZ-2GZ-0S	2013
Misr -1	OASIS/SKAUZ//4*BCN1312*PASTOR.CMSSOOYO1881T-050M-030Y-030M-030WGY-33M-0Y-0S.	2011
Misr -2	SKAUZ/BAV92. CMSS96M03611S-1M-010SY010M-010SY-8M-0Y-0S.	2011
Sakha-95	POSTOR//SITE/MO/3/CHEN/AEGILOPS/SQUARROSA(TAUS)	2018

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% [29]. The host response assessment also included recording the infection type (IT), according to [30]: 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) [30]. 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 [31], as follows:

AUDPC = D [1/2 (Y1 + YK) + Y2 + Y3 +…. Y (K-1)]

Where:

D = Time interval (days between consecutive records);

Y1 + YK = Sum of the first and final disease scores;

Y2 + Y3 + …. + Y (K-1) = Sum of all in-between disease scores.

The rate of rust disease increase (r-value) was also estimated as a function of time, according to the formula by [32]:

$$\text{r-value} = \frac{1}{t_2 - t_1}\left(\log_e \frac{X_2}{1 - X_2} - \log_e \frac{X_1}{1 - X_1}\right)$$

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 variance (ANOVA) over the two seasons was carried out to determine significance differences among cultivars (Table 2), as outlined by [33]. Mean comparisons for variables were made among genotypes using least significant difference (LSD) tests at α = 0.05.

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 μl^{-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 [34,35,36; 37,38, 39, 40, 14, 18, 41, 42, 43, 44, 45,36]. The polymerase chain reaction (PCR) mixture consisted of 20–50 ng of genomic DNA, 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTP, 0.5 μM primer, and 1 U Taq polymerase, in a volume of 0.025 cm^3. 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°C–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.

Table 2
SSR primers, sequences, annealing temperature, expected amplicon size, and linked resistance gene(s) used for detecting variation in 13 wheat cultivars

Marker	Sequences	Annealing temperature	Expected [a] size (bp)	Linked resistant gene(s)
Barc64-7A	GCGGAGTCTGCAATTAGTATAGGTAT GCATCCACCTCCGCAGTCAGT	55	269	Lr
Barc104-6A	GCGCTTCCAAGGCTTAGAGGCT GCGAGCATCAATAATTGAGAAATACATAGA	50	177	Sr13
Barc130-5D	CGGCTAGTAGTTGGAGTGTTGG ACCGCCTCTAGTTATTGCTCTC	52	225	Lr70
Barc1473B	GCGCCATTTATTCATGTTCCTCAT CCGCTTCACATGCAATCCGTTGAT	52	105	Yr?
Barc152-1B	CTTCCTAAAATCGGGCAACCGCTTGTTG GCGTAATGATGGGAGTGGCTATAGGGCAGTT	50	145	Sr33
Barc167-2B	AAAGGCCCATCAACATGCAAGTACC CGCAGTATTCTTAGTCCCTCAT	50	255	Lr
Barc173-6D	GGGGATCCTTCAACAATAACA GCGAGATGGCATTTTTAAATAAAGAGAC	50	237	SrTA10187
Barc180-3B	GCGATGCTTGTTTGTTACTTCTC 3' 5' GCGATGGAACTTCTTTTTGCTCTA	52	194	Yr78
Barc181-1B	CGCTGGAGGGGGTAAGTCATCAC 3' CGCAAATCAAGAACACGGGAGAAAGAA	58	185	Yr26
Barc182-7B	CCATGGCCAACAGCTCAAGGTCTC CGCAAAACCGCATCAGGGAAGCACCAAT	58	105	Lr
Barc183-2B	CCCGGGACCACCAGTAAGT GGATGGGGAATTGGAGATACAGAG	58	179	Sr42
Barc198–6B	CGCTGAAAAGAAGTGCCGCATTATGA CGCTGCCTTTTCTGGATTGCTTGTCA	50	145	Yr78
Barc200-2B	GCGATATGATTTGGAGCTGATTG GCGATGACGTTAGATGCGGAATTGT	52	168	Sr Yanac
Barc352-4D	CCCTTTCTCGCTCGCCTATCCC CTGTTTCGCCCAATCTCGGTGTG	63	249	Lr34/Yr18
Wmc27-2B	AATAGAAACAGGTCACCATCCG TAGAGCTGGAGTAGGGCCAAAG	61	389	Sr P14666
Wmc44-1B	GGTCTTCTGGGCTTTGATCCTG TGTTGCTAGGGACCCGTAGTGG	61	242	Lr49/Yr29
Wmc166-2D	ATAAAGCTGTCTCTTTAGTTCG GTTTTAACACATATGCATACCT	15	305	Yr18
Wmc169-3A	TACCCGAATCTGGAAAATCAAT TGGAAGCTTGCTAACTTTGGAG	61	167	Sr35
wmc175-3A	GCTCAGTCAAACCGCTACTTCT CACTACTCCAATCTATCGCCGT	61	253	Sr9/Yr5
Wmc219-4A	TGCTAGTTTGTCATCCGGGCGA CAATCCCGTTCTACAAGTTCCA	61	204	Sr ND6-43/Sr60

[a] Expected size (bp) on Chinese Spring.

Marker	Sequences	Annealing temperature	Expected [a] size (bp)	Linked resistant gene(s)
Wmc233-5D	GACGTCAAGAATCTTCGTCGGA ATCTGCTGAGCAGATCGTGGTT	61	260	Lr70

[a] Expected size (bp) on Chinese Spring.

Data handling

The SSR data was scored based on the presence or absence of amplified products for each primer, after excluding the unreproducible 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 thirteen wheat cultivars differed significantly in their responses to yellow, leaf, and stem rust disease, as shown by the phenotypic expression of disease parameters during the 2019/2020 growing season (Table 3 and Supplementary TableS1).

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).

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	
	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	20 S	178.50	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	PR	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	-	578	-	0.294	-	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.

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. 2a). 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. 2b). 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. 2c).

Validation of markers linked to stem rust resistance genes

The SSR marker barc104-6A was linked to the gene *Sr13*. This marker had an amplified band size of 250$_{bp}$ in seven genotypes (Gm-9, Gm-10, Gm-11, Sids-13, Sids-14, 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 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. 3a). 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. 3b). 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. 3c). 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. 3e). 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. 3d).

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, Sids-14, 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 (Gem-12, Sids-14, Giza-168, and Sakha-95) were AUDPC type PR or R. The remaining five cultivars showed no introgression for these markers (Table 4, Fig. 4a). 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. 4b).

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. 4c).

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 5). The AUDPC types for cultivars Gm-12, Sids-14, Giza-171, and Giza-168 were PR and R (Table 5). 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 5). The phenotypic responses to infection by different rusts indicated the presence of additional slow-rusting resistance genes.

Table 4
Response of molecular markers for the detection of rust resistance genes (yellow rust, leaf rust, stem rust, leaf/yellow rust, and stem/yellow rust) in thirte

Rust type	Marker	Expected Resistance genes	Cultivar											
			Gm9	Gm10	Gm11	Gm12	Sids1	Sids12	Sids13	Sids14	Giza168	Giza171	Misr1	Mis
Yellow rust	Barc147	*Yr*	+	+	+	+	+			+		+	+	
	Barc180	*Yr78*				+	+		+		+			
Leaf rust	Barc64	*Lr*	+	+	+	+		-	+	+		+		+
	Barc130	*Lr70*	+	+	+	+	+	+	+	+	+	+	+	+
	Barc167	*Lr*			+					+				
Stem rust	Barc104	*Sr13*	+	+	+				+	+		+		+
	Barc152	*Sr33*	+	+	+	+	+	+	+	+	+	+	+	+
	Barc173	*SrTA10187*	+	+	+	+		+	+	+		+	+	+
	Barc200	*Sr*									+			
	Wmc169	*Sr35*						+	+	+	+			+
Leaf/yellow rust	Barc352	*Lr34/Yr18*		+		+	+	+		+	+			+
	Wmc44	*Lr49/Yr29*	+	+	+		+		+	+				
Stem/yellow rust	Wmc175	*Sr9* and *Yr5*	+	+				+		+	+	+		

Table 5
Slow-rusting resistance genes (*Yr*, *Lr*, *Sr* and *Lr/Yr*) associated with molecular markers, and the corresponding phenotypic AUDPC type of seven wheat cultivars

Cultivar	Yellow rust		Leaf rust		Stem rust		Yellow, Leaf, Stem rust
	AUDPC Type	Expected Resistance genes	AUDPC Type	Expected Resistance genes	AUDPC Type	Expected Resistance genes	Expected Resistance genes
Sakha-95	PR	None	R	*Lr~70*	R	*Sr~33*	*Lr~34/Yr~18*
Gm-12	PR	*Yr~78*	PR	*Lr~70*	R	*Sr~33, TA10187*	*Lr~34/Yr~18*
Sids-14	PR	*Yr~*	PR	*Lr~70*	R	*Sr~13, 33, TA10187*	*Lr~34/Yr~18*, *Lr~49/Yr~29*
Giza-171	R	*Yr~*	PR	*Lr~70*	PR	*Sr~13, 33, TA10187*	None
Giza-168	PR	None	PR	*Lr~70*	PR	*Sr~33, TA10187*	*Lr~34/Yr~18*
Sids-1	PR	*Yr~78*	Sus	*Lr~70*	R	*Sr~33*	*Lr~34/Yr~18*, *Lr~49/Yr~29*
Sids-13	S	None	PR	*Lr~70*	R	*Sr~13, 33, 35, TA10187*	*Lr~49/Yr~29*

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 programs worldwide [46, 47, 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 [48]. 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 [14]. 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 owm45F3R3, 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 [49]. 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* [15]. The gene *Yr30* is linked with the SSR markers xgwm533 and xgwm493 [50, 15]. 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* [17]. 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 [45].

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 [51]. 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 [52]. 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 [53, 54, 55. 56, 18]. The yellow rust resistance genes *Yr64* and *Yr15* are linked with the SSR markers barc8, Xgwm413, and Xgwm273. The presence of fifty F_5 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* [19]. According to [21, 26], the marker xgwm11 amplified a *Yr15*-specific 215_{bp} fragment; the same size band 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 [21].

The leaf rust resistance gene *Lr70*, which has been newly mapped in the common wheat accession KU3198 (36), is linked with the SSR marker barc130. The SSR marker cfd20 is linked with the leaf rust resistance gene *Lrk1* [36]. 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 SCS265512, which is linked to *Lr19*. Of the original 161 plants, 150 were determined to be positive for *Lr19* [23]. Molecular APR markers for the leaf rust resistance gene *Lr48* in wheat were reported by [57]. 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* [57]. [58] 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/r2, 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 [58]. 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 [59]. 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* [60]. 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 F_2 homozygous susceptible plants, and were further genotyped with ten additional markers [25].

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* [38]. The resistance gene *SrTA10171* in the validated population $BC2F_1$ 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 [40]. The development and validation of molecular markers linked with the stem rust resistance gene *Sr13* in durum wheat was described by [35]. 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 [35]. The validation of markers linked to the stem rust resistance gene *Sr28*, which is effective against the race Ug99, was described by [61]. In [43], 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* [61]. [41], 62] 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 [41]. The SSR markers cfd49 and barc183 were found to flank a gene that was assumed to be *Sr42* in wheat genotypes [62]. A detected recombination between *Fhb1* and *Sr2* using molecular markers was reported by [42], [63, 64]. In these studies, UMN10 was a codominant marker (237 and 240_{bp}), whereas csSr2 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 [63].Markers flanking csLV34–Xgwm295 were linked with the *Yr18/Lr34* genes, which confer effectively durable resistance to rust diseases [65, 66] 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 [67]. [68] 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 F_3 wheat lines, 28 were positive for the SSR marker wmc221, indicating the presence of *Lr19/Sr25*. Out of fourteen chosen F_4 lines from F_3, nine were positive for *Lr19/Sr25*. The advanced breeding lines viz., WBM3632 (WBM3697), and WBM3635 were also positive for *Lr19/Sr25* using the SCAR marker SCS265512 [69]. [70] identified a durable molecular marker for the validation of the stem rust resistance gene *Sr45/Lr21* in common wheat. 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 [35]. [24] 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 [24]. The APR genes express resistance at the post-seedling stages, showing non-supersensitive reactions and slow disease expansion in cultivars carrying these genes. This type of resistance has also been referred to as slow-rusting or partial resistance, and is considered more durable than other types of resistance [71]. Some APR genes confer pleiotropic resistance to various diseases, including yellow rust, leaf rust, and stem rust. These include *Yr18/Lr34/Sr57*, *Yr29/Lr46/Sr58*, and *Yr46/Lr67/Sr55* (https://wheat.pw.usda.gov/GG3/wgc). Gene-based and closely linked molecular markers (SNP, STS and CAPS) were used to validate the presence of resistance alleles of the genes *Lr34*, *Lr46*, *Lr67*, *Lr68*, and *Sr2* [72]. [73] studied the molecular breeding of wheat lines for resistance against multiple rusts and Fusarium head blight (FHB), reporting durable resistance against both the rusts and FHB by combining the six resistance genes *Lr19*, *Lr34/Yr18/Sr57/Pm38/Ltn1*, *Sr2/Yr30*, *Sr26*, *Sr39*, and *Fhb1*.

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 5). 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.

Declarations

Funding: The current work was funded by NRCwithin the 12tj research plan projects,through supporting project number (12050142) National Reseach Center,Giza.Egypt.

Conflicts of interest/Competing interests : All authors declare that they have no conflict of interest.

Availability of data and material :available

Code availability : no code

Authors' 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 mange the whole work.

Regulation: the manuscript complies the local and national regulations.

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.

Ethics 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.

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Figures

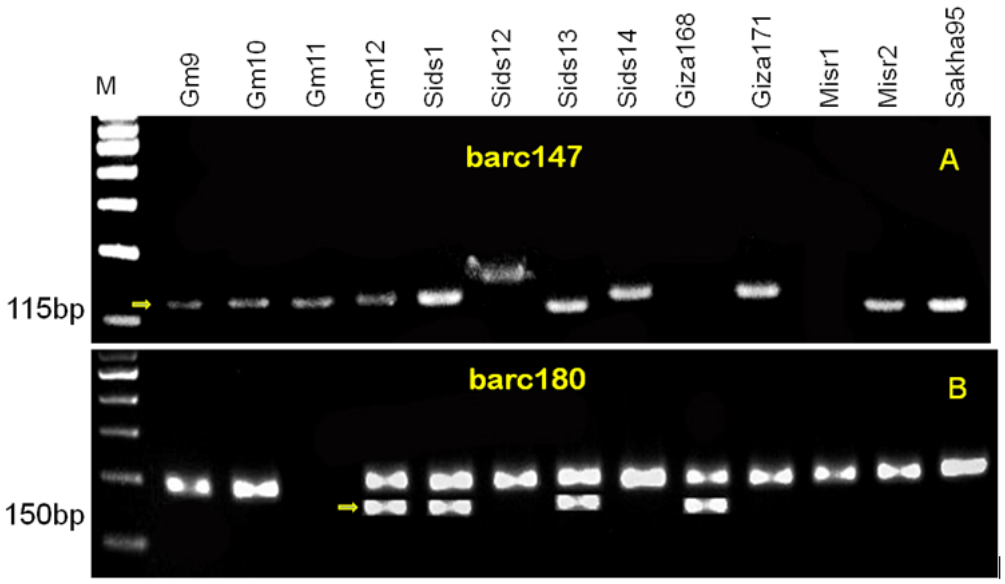

Figure 1

Agarose gel electrophoresis showing allele size of the SSR markers A barc147 and B barc180 in thirteen wheat cultivars

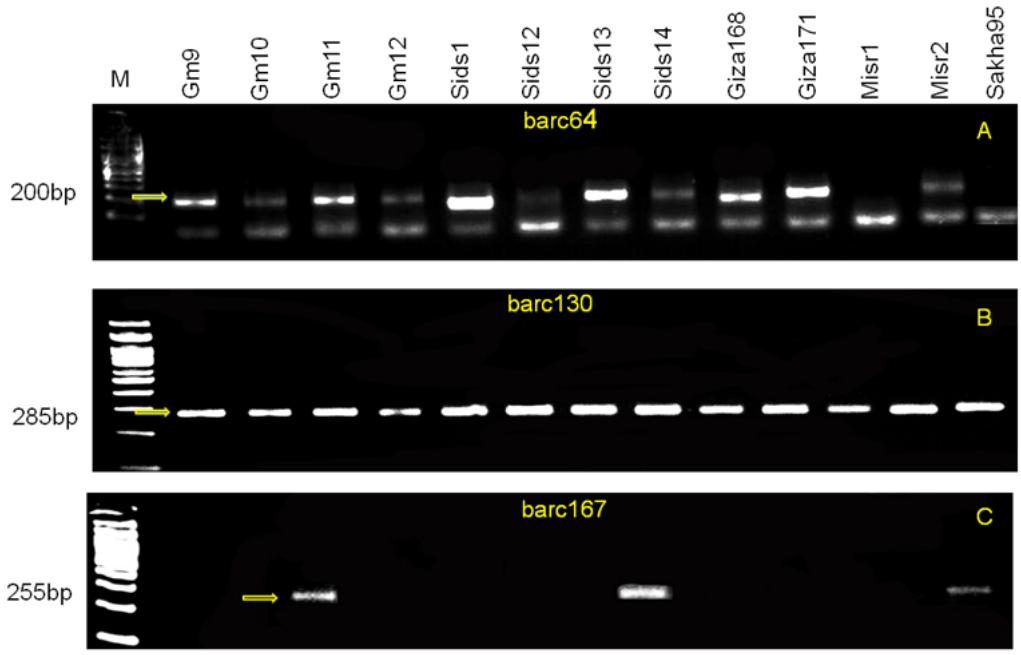

Figure 2

Agarose gel electrophoresis showing allele size of the SSR markers A barc64, B barc130, and C barc167 in thirteen wheat cultivars

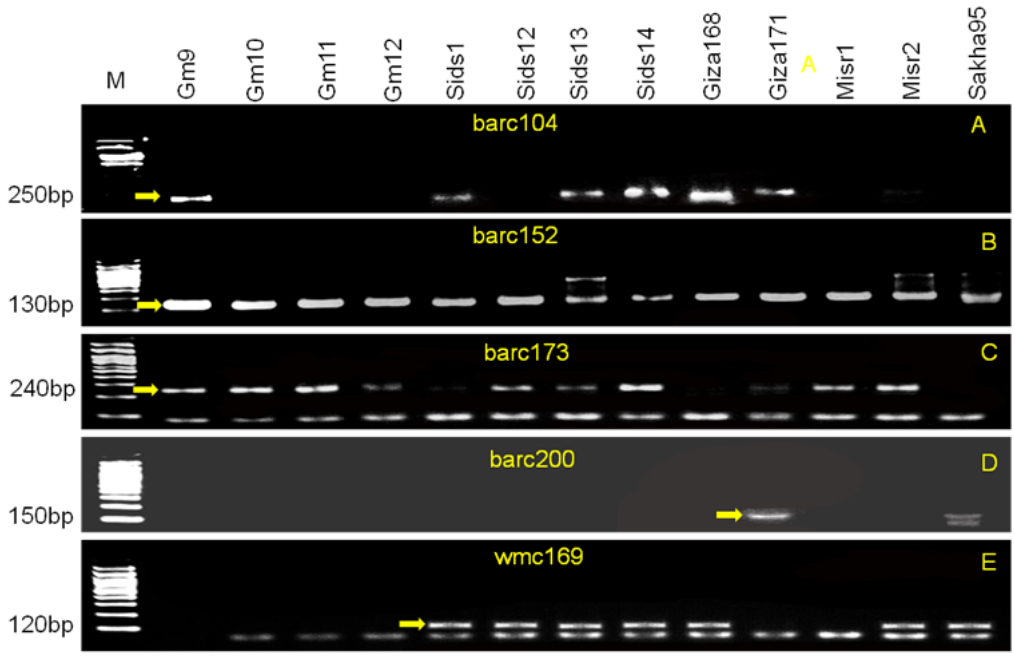

Figure 3

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

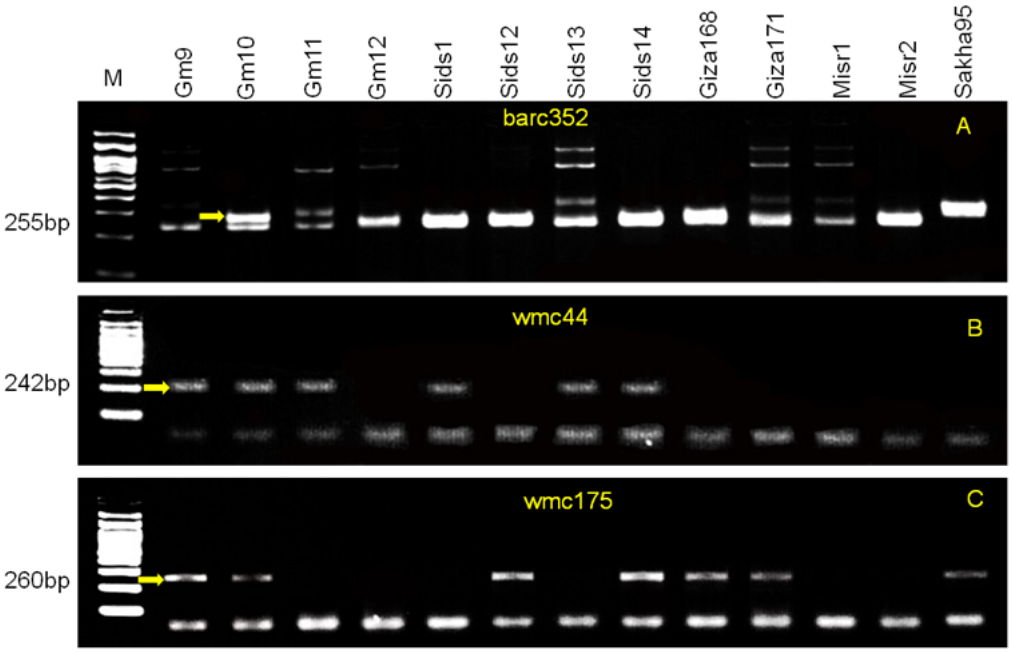

Figure 4

Agarose gel electrophoresis showing allele size of the SSR markers A barc352, B wmc44, and C wmc175 in thirteen wheat cultivars

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