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Molecular identification of yellow rust resistance genes in some wheat and triticale cultivars and their resistance to *Puccinia striiformis* f.sp. *tritici*

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Abstract: Yellow rust (YR), caused by *Puccinia striiformis* f.sp. *tritici* (*Pst*), is a global threat to wheat production. In this study the response of 46 wheat and triticale cultivars to *Pst* at the adult plant stage (APS) was evaluated during two successive growing seasons at Sulaimania, Iraq. Also, we used a molecular analysis to find the yellow rust resistance (*Yr*) genes present in the individual cultivars. The results revealed large differences in the response to *Pst* between the cultivars. Most of the cultivars were susceptible to YR; the mean coefficients of infection (CI) varied from 0.23 in cv. Sarah to 83.33 in Hsad. High resistance levels were found in Al-Wand, Kalar 1, Rezan, and Sarahat APS, while Al-Rashid, Charmo, Faris 1, Maaroof, Rabiea, and Iratom displayed moderate resistance. The level of Yellow rust infection was higher in 2023 than in 2022 in most tested cultivars. Molecular analysis revealed the highest number of *Yr* genes (*Yr2*, *Yr5*, *Yr7*, *Yr9*, *Yrvav*, *Yr15*, *Yr24*, *Yr26*, and *Yr32*) in the cv. Al-Wand, followed by Sulaimani 2 with eight *Yr* genes (*Yr2*, *Yr5*, *Yr7*, *Yr9*, *Yr15*, *Yr24*, *Yr26*, and *Yr32*). Only one *Yr* gene was found in Iratom and Tamuz 3. *Yr2* was the most frequently identified gene, present in the majority of tested cultivars (87%), followed by *Yr7* (76%) and *Yr9* (74%), respectively.

Keywords: DNA extraction; PCR; stripe rust; *Triticum aestivum*; *Yr* genes

Wheat (*Triticum aestivum* L.) provides 20% of human nutrition calories and protein, which feeds 36 to 40% of the world's population (Porras et al. 2022). Iraq currently cultivates 2.4 Mha of wheat (Al-Maaroof 2022). Fungal diseases are the main cause of wheat grains and quality decrease (Al-Maaroof 2022). Yellow rust (YR), incited by *Puccinia striiformis* f.sp. *tritici* (*Pst*), significantly affects wheat in temperate zones (Bux et al. 2012). The distribution of yellow rust was formerly restricted to specific wheat fields in Iraq's northern area. But it has moved to the central and southern regions since 1988. Under normal circumstances, yellow rust can lead to substantial losses of 30–50% in commercial wheat production (Al-Maaroof 2022). About 88% of the world's wheat cultivars are susceptible to YR (Zhang et al. 2022). According to estimates, YR cost the globe at least $1 billion annually and 5.5 million tonnes of yield (Beddow et al. 2015). The disease affects the number, size, and weight of grains, in addition to spikes in number (Gulmorodov 2023). Moreover, seed vigour and emergence from infected plants are reduced. YR can lead to grain losses of 10–70%, contingent on the cultivar susceptibility, primary infection time, and disease development rate (Al-Maaroof & Nori 2018).

A series of regional YR outbreaks have been reported in Central West Asia and North Africa (CWANA) over the last two decades (Ali et al. 2017). These epidemics have been attributed to the rapid spread of newly emerged *Pst* races, which significantly

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reduced wheat yield (Hovmøller et al. 2011). The new races were more aggressive and heat tolerant compared to the older races (Milus et al. 2009). Since 2019, most of Iraqi wheat farms have been severely plagued by disease pressure, particularly in the years 2009, 2010, 2013, 2016 and 2019. The majority of wheat fields showed significant disease incidence (DI) and disease severities (DS) during the two worst YR epidemics, which occurred in 2010 and 2019 (Al-Maaroof 2022). The enormous effort made by Iraqi breeding programs to increase YR resistance, which was successful in releasing a number of resistant cultivars, was significantly impacted by the new *Pst* races, *PstS2* and *PstS2*, *v27* that were able to overcome the resistance of these cultivars (Al-Maaroof et al. 2020a, b, 2022).

Developing of resistant cultivars requires constant observation for virulence change, as *Pst* can quickly grow into new races and disperse by air over long distances (Chen et al. 2002). YR can be managed by combining disease resistance, fungicide applications, and agricultural practices. However, certain cultural practices might not be beneficial when used alone (Chen 2005). Genetic resistance is critical to any breeding program that aims to increase YR resistance (Almajidy et al. 2017). It is a cost-effective and eco-friendly method for YR control. There are two types of resistance: seedling resistance (SR) and adult plant resistance (APR). According to Basnet (2012), SR is typically conferred by a single gene at all plant growth stages, but APR is generally conferred by many genes later in the growth stages (Bhavani et al. 2022). About 85 identified *Yr* genes have been recognized in wheat including, 28 APR and 55 SR genes (Saleem et al. 2022). The majority of *Yr* genes shows race SR, while *Yr18*, *Yr29*, *Yr30*, *Yr32*, *Yr36*, *Yr39*, *Yr46*, *Yr48*, *Yr49*, *Yr52*, *Yr54*, *Yr59*, and *Yr62* showed APR (Rahmatov et al. 2019). Currently, only ten *Yr* genes have been cloned (*Yr5/YrSP*, *Yr7*, *Yr10*, *Yr15*, *Yr18*, *Yr36*, *Yr46*, *YrAS2388* and *YrU1*). Every cloned gene belongs to a different protein family (Tene et al. 2022). The study aimed to assess wheat and triticale cultivars' response to *Pst* and molecular identification of the available *Yr* genes in each cultivar.

MATERIAL AND METHODS

Response of some wheat and triticale cultivars to *Pst*. Field trails were conducted for two crop seasons (2021–2023) at Bakrajo (latitude N 35° 32 036; longitude E 045° 21 818). Forty six bread wheat and triticale cultivar's responses to YR were assessed in the field at APS (Table S1 in Electronic Supplementary Material (ESM)). Seeds of each cultivar were sown in 4 rows in 2 m^2 plots, with blocks spaced in 1 m apart and rows and plots separated by 20 and 50 cm, respectively. Three replications of randomised complete block designs (RCBD) were used in the study. Two rows of YR susceptible cultivars (Hsad and Morocco) were sown throughout the trail area to ensure artificial inoculation of the tested cultivars. Artificial inoculation of the spreader plants was conducted by spraying uredospores suspension of race *PstS14* at the booting stage. The virulence/avirulence pattern of this race was *Yr2*, *Yr3*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr25*, *Yr32*, *YrSp* and *Avocet S/Yr1*, *Yr4*, *Yr5*, *Yr10*, *Yr15*, *Yr24* and *Yr27*. The plants were sprayed with water three times per week before sunset based on whether it rained, from late March to mid-May, in order to promote the rust epidemic.

Disease scoring. YR development on the tested cultivars was closely monitored in the field. Disease severity and infection type (IT) of YR were assessed according to (Lewellen et al. 1967). Coefficient of infection (CI) was calculated by multiplying the DS with a fixed value of host response coefficient (Periyannan 2017). Data were analysed by JIM software (SAS Institute Inc., USA) to determine the source of variance. Duncan's multiple range test at a 5% probability level was used to compare the trait means.

Molecular identification of the available *Yr* genes in wheat and triticale cultivars. Seeds of 44 wheat and 2 triticale cultivars were surface sterilised by 70% ethanol for 10 s, then washed for 10 min. in 20% commercial bleach and twice rinsed with ddH_2O. Fresh leaves of 2-leaf seedlings were used to extract the genomic DNA using the Addbio DNA extraction protocol (Al-Maaroof & Salih 2022) and the Prep Genomic DNA Extraction Kit (Yuseong-gu, Daejeon, South Korea).

DNA quantification and purification. DNA quality and quantity assessments, ethidium bromide staining, and 0.8% agarose gel electrophoresis were carried out with a spectrophotometer. Utilizing a gel documentation system (Sygene, UK) for photo capture, the bands were visualised using UV light. The DNA concentration was measured in ng/L using a NanoDrop Lite Spectrophotometer (Thermo Scientific, USA). As per Chen et al. (2004), the value of nucleic acid concentration in the study varied from 53 to 313 ng/µL, while the purity levels degrees

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varied from 1.3 and 2.0 ng. The isolated genomic DNA was kept at –20 °C until use.

Primer design and preparation. Common SSR and STS primers were selected from the grain genes database (www.graingenes.org) in line with published microsatellite primers (Somers et al. 2004) using the parameters listed in Table 1. The primers were then used to amplify the genomic DNA. 10 pmol µL of the primers were prepared by diluting the primer solution with 1–9 ddH_2O after adding 300–320 µL ddH_2O, the mixture was mixed several times with a pipette. The primers were stored at –20 °C until use.

PCR amplification and gel electrophoresis. Five µL of template DNA and 10 µL master mix (add *Taq* Master at 2× concentration). The 20 µL total volume of the DNA amplification reaction contained 20 mM of Tris HCl (pH8.8), 1 mM KCl, 0.2% Triton® X100, 4 mM $MgCl_2$ protein stabiliser, sediment, loading dye and 0.5 mM each of dATP, dCTP, dGTP, and dTTP, 1 µL (100 pmol/µL) of each SSR-derived primer pair (forward and reverse), 3 µL ddH_2O, vertex places and mixed many times before setting the mixture into the PCR machine. The following were the PCR conditions: the first denaturing phase 5 min at 94 °C; denaturation: 45 cycles of 50 s at 94 °C; annealing: 52.3 to 67 °C. Denaturation options include the following: 50 s of denaturation at 72 °C followed by the last extension phase lasts 10 min at 72 °C (Rani et al. 2019). 1% agarose gel was used for electrophoresis, and 6 µL (0.5 µg/mL) of ethidium bromide solution was used for staining. A 100 bp DNA ladder was placed into one well, and each well contained 5 µL of PCR product. The electrophoresis power supply was set to (90 W, 800 mA, 85 volts) for 1–2 h. Once the migration was completed, the power supply was shut off, and the amplified DNA bands or gel were removed and analysed under a UV lamp. Band sizes were determined by means of comparison with a 100 bp DNA ladder from MBI Fermentas, USA. Potential polymorphisms between bulks were confirmed by repeated amplification.

RESULTS AND DISCUSSION

Response of some wheat and triticale cultivars to *Pst*. Table 2 displays different responses of the tested cultivars against *Pst* over the course of two successive growing seasons 2021 to 2023. Significant differences between the cultivars in both seasons were revealed by the CIs. variance analysis showed significant differences between the cultivars in both seasons. The combined study also revealed substantial differences among the cultivars, years and their interactions, suggesting distinct responses of the cultivars to YR. Seven groups can be used to categorize the cultivars. Al-Wand, Kalar 1, Rezan, and Sarah (8.7% of the cultivars) make up the resistant group (G1). The mean DS and CI of G1 varied between cv. Sarah's 1.2% and 0.23 and Al-Wand 5.8% and 1.2. The resistant (R) to moderate resistant (MR) group (G2) comprise Al-Rashid, Charmo, Faris 1, Maaroof, Rabia, and Iratom (13%). The DS and CI for this group ranged from 2.5 to 17%, and 0.8 to 5.1 in Iratom and Al-Rashid, respectively. No significant differences were detected between G2 and G1 members' means. Hawler 2 only represents G3 (2.2%), which explored MR with 7% DS and 2.8 CI. Only Azmar2, which displayed MR in the second season

Table 1. Target gene, primer name, location in the chromosome, expected band size and annealing temperature of the *Yr* genes

Gene	Primer name	Locus on chromosome	Expected band size (bp)	Reference	Annealing temperature (°C)
Yr2	*wmc364*	7B	190–204	Rani et al. (2019)	60.3
Yr5	*Xgwm501*	2B	166–172	Kim et al. (2020)	60
Yr7	*Xgwm526*	2B	140–160	Kim et al. (2020)	60
Yr9	*Xgwm582*	1BS	150	Rani et al. (2019)	52.3
Yr10/Yrvav	*Xpsp3000*	1BS	260–285	Tahir et al. (2020)	61
Yr15	*Xgwm273*	1BS	156–200	Tahir et al. (2020)	58
Yr17	*VENTRIUP/LN2*	2AS	259	Kim et al. (2020)	64
Yr18	*Xgwm295*	7DS	160–250	Kim et al. (2020)	60
Yr24/26	*Barc181*	1BS	180–220	Kim et al. (2020)	67
Yr32	*wmc198*	AD	159–160	Rani et al. (2019)	56

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Table 2. Mean disease severity, infection types, and coefficient of infection of some wheat and triticale cultivars against yellow rust during two successive seasons 2021–2023 at Bakrajo, Sulaimania, Iraq

Cultivar name	2021–2022			2022–2023			Mean		
	DS%	IT	CI	DS%	IT	CI	DS%	IT	CI
Hsad	73.3	S	73.3^{a}	93.3	S	93.3^{ab}	83.3	S	83.3^{a}
Tamuz 3	63.3	S	63.3^{ab}	100	S	100^{a}	81.7	S	81.7^{a}
Bhuth 158	43.3	S	43.3^{a-e}	98.3	S	98.3^{a}	70.8	S	70.8^{ab}
Ashur	50.0	S	50.0^{a-d}	86.7	S	86.7^{a-c}	68.3	S	68.3^{ab}
Baraka	40.0	S	40.0^{a-e}	93.3	S	93.3^{ab}	66.7	S	66.7^{a-c}
SaberBeg	33.3	S	33.3^{a-e}	100	S	100^{a}	66.7	S	66.7^{a-c}
Bhuth 10	45.0	S	45.0^{a-e}	90.0	S	90.0^{a-c}	67.5	S	67.5^{a-c}
Tamuz 2	38.3	S	38.3^{a-e}	91.7	S	91.7^{a-c}	65.0	S	65.0^{a-c}
Al-Madaen	33.3	S	33.3^{a-e}	96.7	S	96.7^{a}	65.0	S	65.0^{a-c}
Fath 1	36.7	S	36.7^{a-e}	86.7	S	86.7^{a-c}	61.7	S	61.7^{a-d}
Bhuth 22	25.0	S	25.0^{b-e}	91.7	S	91.7^{a-c}	58.3	S	58.4^{a-e}
Latifia	35.0	S	35.0^{a-e}	75.0	S	75.0^{b-e}	55.0	S	55.0^{a-f}
Bengal	43.3	S	43.3^{a-e}	66.7	S	66.7^{d-g}	55.0	S	55.0^{a-f}
Qandaharia	28.3	S	28.3^{a-e}	73.3	S	73.3^{c-f}	50.8	S	50.8^{a-f}
Uruk	25.0	S	25.0^{b-e}	75.0	S	75.0^{b-d}	50.0	S	50.0^{a-f}
Iba 99	13.3	S	13.3^{c-e}	86.7	S	86.7^{a-c}	50.0	S	50.0^{a-f}
Cham 6	16.7	S	16.7^{b-e}	73.3	S	73.3^{c-f}	45.0	S	45.0^{b-g}
Hawler 4	16.7	S	16.7^{b-e}	56.7	S	56.7^{d-i}	36.7	S	36.7^{b-h}
Wafia	26.7	S	26.7^{a-e}	36.7	S	36.7^{j-m}	31.7	S	31.7^{c-h}
Sulaimani 2	16.7	MS	13.3^{c-e}	35.0	S	35.0^{k-n}	25.8	MS-S	23.3^{e-h}
Kalar 2	15.0	MS	12.0^{c-e}	11.0	S	11.0^{r}	13.0	MS-S	11.7^{gh}
Alaa	50.0	MS	40.0^{a-e}	81.7	MS	65.4^{d-h}	65.8	MS	52.7^{a-f}
Al-Nur	68.3	MS	54.6^{a-c}	60.0	MS	48.0^{h-k}	64.2	MS	51.4^{a-f}
Babil 113	45.0	MS	36.0^{a-e}	66.7	MS	53.3^{g-j}	55.8	MS	44.7^{b-g}
Iba 95	30.0	MS	24.0^{b-e}	73.3	MS	58.6^{d-i}	51.7	MS	41.3^{b-g}
Baghdad 3	41.7	MS	33.3^{a-e}	36.7	MS	29.3^{k-q}	39.7	MS	31.3^{c-h}
Abu Ghraib	10.0	MS	8.0^{c-e}	60.0	MS	48.0^{h-l}	35.0	MS	28.0^{d-h}
Iraq	34.0	MS	27.2^{a-e}	22.3	MS	17.9^{n-r}	28.2	MS	22.5^{e-h}
Al-Eiz	14.3	MS	11.5^{c-e}	41.7	MS	33.3^{k-n}	28.0	MS	22.4^{e-h}
Adana	12.3	MS	9.9^{c-e}	40.0	MS	32.0^{k-o}	26.2	MS	20.9^{f-h}
Bura	6.7	MS	5.36^{de}	40.0	MS	32.0^{k-p}	23.3	MS	18.7^{f-h}
Barcelona	11.7	MS	9.3^{c-e}	18.3	MS	14.7^{o-r}	15.0	MS	12.0^{gh}
Al-Samawa	15.0	MS	12.0^{c-e}	14.0	MS	11.2^{r}	14.5	MS	11.6^{gh}
Baghdad 1	11.7	MS	9.3^{c-e}	12.3	MS	9.9^{r}	12.0	MS	9.6^{gh}
Azmar	14.0	MR-MS	8.4^{c-e}	56.7	MS	45.3^{i-m}	35.3	MR- MS	21.2^{f-h}
Hawler 2	7.3	MR	2.9^{de}	6.7	MR	2.7^{r}	7.0	MR	2.8^{h}
Al-Rashid	12.3	R-MR	3.7^{de}	21.7	R-MR	6.5^{r}	17.0	R-MR	5.1^{h}
Charmo	13.3	MR	5.3^{de}	16.7	R-MR	5.0^{r}	15.0	R-MR	4.5^{h}
Faris 1	13.3	MR	5.3^{de}	9.0	R-MR	2.7^{r}	11.2	R-MR	3.4^{h}
Maaroof	6.7	R	1.3^{e}	15.0	R-MR	4.5^{r}	10.8	R-MR	3.3^{h}
Rabiea	9.7	MR	3.9^{de}	2.3	R-MR	0.7^{r}	6.0	R-MR	1.8^{h}
Iratom	3.3	MR	1.3^{e}	1.7	R	0.3^{r}	2.5	R-MR	0.8^{h}

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Table 2 to be continued

Cultivar name	2021–2022			2022–2023			Mean		
	DS%	IT	CI	DS%	IT	CI	DS%	IT	CI
Al-Wand	3.6	R	0.7^{e}	8.0	R	1.6^{r}	5.8	R	1.2^{h}
Kalar 1	6.7	R	1.3^{e}	0.3	R	0.1^{r}	3.5	R	0.7^{h}
Rezan	6.7	R	1.3^{e}	0.0	R	0^{r}	3.3	R	0.7^{h}
Sarah	2.3	R	0.5^{e}	0.0	R	0^{r}	1.2	R	0.2^{h}
Mean	25.9	R-S	22.3^{b-e}	50.5	R-S	46.5^{i-m}	38.0	R-S	34.3^{b-h}

DS – disease severity; IT – infection type; R – resistant; MR – moderately resistant; MS – moderately susceptible; S – susceptible (Lewellen et al. 1967); CI – coefficient of infection, calculated by multiplying the DS value with IT (Periyannan 2017); each number is a mean of three replicates, numbers followed by the same symbols significantly are not different at 0.05 level according to Duncan's multiple test analysis

and MR to moderately susceptible (MS) in the first, is included in G4. Azmar had a DS of 35.3% and CI of 21.2. G5 represent the second largest group (28.3%) with MS, which includes Alaa, Al-Nur, Babil 113, Iba 95, Baghdad 3, Abu Ghraib, Iraq, Al-Eiz, Adana, Bura, Barcelona, Al-Samawa and Baghdad 1. The DS of G5 varied from 12–65.8%, with a mean CI ranging from 9.6 in Baghdad 1 to 52.7 in Alaa. Sulaimani 2 and Kalar 2, which have an MS to susceptible (S) response with CIs of 11.7 to 23.3 and 13–25.8% DS are present in G6 (4.4%). G7 comprise the biggest group (41.3%), which is composed of 19 S cultivars, Hsad, Tamuz 3, Bhuth158, Ashur, Baraka, SaberBeg, Bhuth 10, Tamuz 2, Al-Madaen, Fath 1, Bhuth 22, Latifia, Bengal, Qandaharia, Uruk, Iba 99, Cham 6, Hawler 4 and Wafia. The group's mean DS and CI varied from 31.7 % in cv. Wafia to 83.3% in Hsad.

Host-parasite interaction of *Pst* and the susceptible cultivars is maintained during both seasons. With the exception of Sulaimani 2 and Kalar 2, which were classified as MS in 2022 but as S in 2023. They also explored higher DS and CI in 2023. Additionally, there were no differences in the IT of the G5 MS cultivars in both years. In 2022, Azmar's, IT switched from MR-MS to MS in 2023 with a minor increase in DS and CI. Hawler 2 explored the same IT in 2023 with a minor increase in DS and CI. Wheat cvs Charmo, Faris 1, Maaroof, Rabiea, and Iratom displayed distinct ITs in both seasons, with a minor increase in DS and CI in the second season. Except for Charmo and Faris 1, Rabiea displayed MR in 2022 but switched to R-MR in 2023. Conversely, Maaroof's IT changed from R in 2022 to R-MR in 2023 and Iratom went from MR in 2022 to R in 2023. Despite a large increase in DS and CI of all studied cultivars in the second season, the ITs of G1 cultivars stayed constant in both seasons. The high incidence of YR in the susceptible cultivars during the second season may have been caused by favourable climatic factors and higher rainfall at the test location.

These results agree with those of Al-Maaroof et al. (2020b), where comparable outcomes were observed for the HS reaction for SaberBeg, Latifia, and Bengal and S reaction for Ashur, Baraka, Bengal, Hawler 4, Tamuz 2, Al-Madaen, Cham 6, Fath 1, Bhuth 22 and Wafia. Other cultivars have different IT: Bhuth 158, Bhuth 10, Latifia, Uruk, Iba 99, Hsad and Tamuz 3, have distinct ITs. Additionally, while Sulaimani 2 and Kalar 2 had S reaction, the study findings showed MS-S. Alaa, Al-Nur, Babil 113, Iba 95, Baghdad 3, Abu Ghraib, Iraq, Al-Eiz, Adana, Bura and Baghdad 1 yielded identical results.

The genetic basis of resistance to YR in Iraqi wheat cultivars was greatly strengthened by introducing YR-resistant genes from sources in global collections into breeding programs using local cultivars (Al-Maaroof 2022). The creative (Al-Maaroof & Nori 2018) led to the development of the novel YR-resistant cultivars Maaroof and Charmo. These results also align with those of (Al-Maaroof et al. 2015), which found that across a range of farmer fields, wheat cultivars Buhoth 22, Barcelona, Cham 6, Iba99, Aras, SaberBeg, and Abu Ghraib were HS to YR. Al-Maaroof et al. (2020b) assessed the YR performance of several wheat cultivars from 2014 and 2016. He found that whereas Maaroof and Azmar exhibited high level of resistance Alaa, Al-Wand, and Charmo displayed MR, Hsad showed MS, whilst SaberBeg, Aras, and Adana displayed HS. Earlier studies also noted SaberBeg, Tamuz-2, and Adana susceptibility as well as the multiple resistance of Maaroof to YR, LR and SR diseases besides its high yield potential (Al-Maaroof et al. 2020a).

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The genetic makeup of wheat cultivars affects both their resistance levels and YR responses. There may be variations in the IT of certain cultivars due to the emergence of new *Pst* races, particularly PS14, which was identified in 2023 with virulence against the resistant genes *Yr2*, *Yr3*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr25*, *Yr32*, *YrSp* and *Avocet S* at seedling stage under control conditions. While certain cultivars are durable resistant, it's likely that it will eventually turn into S. For instance, Al-Ize was initially categorized as MR in 1995; however, after a decade, it was reclassified as S (Al-Maaroof et al. 2015). However, Tamuz 2 was first released in 1992 with an R classification before being changed to S (Al-Maaroof & Nori 2018). The R of Al-Wand, Kalar 1, Rezan, and Sarah, as well as the R-MR of Maaroof, Al-Rashid, Charmo, Faris 1, Rabiea, and Iratom, may be attributed to the multiple resistant genes in their genetic backgrounds. No other S cultivars possessed these genes.

The high CI levels in the S cultivars, which varied from 73.3 in 2022 to 93.3 in 2023 in Hsad, may be due to the availability of virulent *Pst* races and S cultivars, along with favourable environmental conditions for YR development. The CI value facilitates statistically comparing genotypes with various YR responses.

Pst can grow into new aggressive and virulent races through sexual reproduction, recombination, mutation and other mechanisms. Therefore, monitoring the *Pst* population is crucial to identify any new virulence. The new races may be able to overcome some cultivars' resistance based on the boom and bust cycle (Periyannan 2017). Numerous research have demonstrated *Pst*'s ability to develop new races (Bartoš et al. 2000). Furthermore, various environmental conditions in field studies may yield different results in subsequent tests (Zhang et al. 2022).

The ideal time for YR development was between the first of March and the end of May in both seasons, when the mean temperature was between 5–25 °C. However, the ideal temperature ranges for YR emergence, development, and spread were 4.5–31.8 °C and 5.9–27.7 °C, respectively in this study (Figure S1 in ESM). This result validates 0–23 °C in the germination range of *Pst* urediniospores. 12–15 °C and 5–20 °C are the ideal temperature for fungal growth and sporulation, respectively. Infection can occur at minimum and maximum temperatures of 2 °C and 23 °C. The temperature range was ideal in 2023. Furthermore, the humid weather of the season was ideal for YR development. Spore germination is promoted by high relative humidity (Chen 2005). Figure S1 in ESM shows that the average amount of precipitation for the seasons 2021/22 and 2022/23 was 436.4 and 718.8 mm, respectively. As a result of 2023's higher relative humidity, higher precipitation rate, and better monthly rainfall distribution overall contributed to increased DS, IT and CI.

Molecular identification of the available *Yr* genes in wheat and triticale cultivars. The study investigated the identification of 12 *Yr* genes in 46 wheat and triticale cultivars using 10 *Yr* gene-linked markers. The QTLs were found on chromosomes 7B, 2B (2), 1BS (4), 2AS, 7DS, and 2AL. The markers were successful in identifying the particular *Yr* genes in the tested cultivars. The available *Yr* genes in the tested cultivars are listed in Table 3. In different wheat cultivars *Yr2*, located on chromosome 7B, is efficient against certain *Pst* races (Rani et al. 2019).

But as can be shown in Figure 1 and Table 3, marker *wmc364* yield a band size of 190–204 bp in most cultivars, except Alaa, Hsad, Wafia, Al-Samawa, Iratom, and Al-Eiz. According to Al-Maaroof et al. (2003), *Pst* races *6E16* and *230E150* were virulent on a few genes, including *Yr2* in Iraq. *Yr2* has been ineffective against most of the widespread new races, including those that are common in Iraq (Al-Maaroof et al.

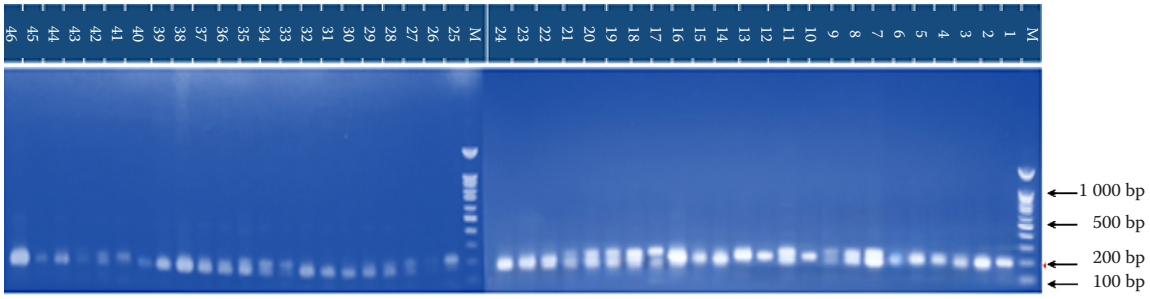

Figure 1. SSR amplification profile for primer *wmc364* (*Yr2*) on 46 wheat cultivars
M – 100 bp DNA ladder; the numerical designation of the genotypes is shown in Table 3

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Table 3. Identification of the available *Yr* genes in various wheat and triticale cultivars using molecular markers

No.	Cultivar name	*wmc364* (*Yr2*)	*Xgwm501* (*Yr5*)	*Xgwm526* (*Yr7*)	*Xgwm582* (*Yr9*)	*Xpsp3000* (*Yr10*)	*Xpsp3000* (*Yrvav*)	*Xgwm273* (*Yr15*)	*VENTRIUP/LN2* (*Yr17*)	*Xgwm295* (*Yr18*)	*Barc181* (*Yr24*)	*Barc181* (*Yr26*)	*Wmc198* (*Yr32*)	Detected *Yr* gene
1	Al-Wand	+	+	+	+	–	+	+	–	–	+	+	+	*Yr2, Yr5, Yr7, Yr9, Yrvav, Yr15, Yr24, Yr26, Yr32*
2	Sulaimani 2	+	+	+	+	–	–	+	–	–	+	+	+	*Yr2, Yr5, Yr7, Yr9, Yr15, Yr24, Yr26, Yr32*
3	Hawler 2	+	–	+	–	–	–	–	–	–	+	+	–	*Yr2, Yr7, Yr24, Yr26*
4	Hawler 4	+	–	–	+	+	–	–	–	–	+	+	–	*Yr2, Yr9, Yr10, Yr24, Yr26*
5	Azmar	+	–	+	+	–	–	–	–	–	+	+	–	*Yr2, Yr7, Yr9, Yr24, Yr26*
6	Alaa	–	–	+	–	–	–	–	–	–	+	+	–	*Yr7, Yr24, Yr26*
7	Baraka	+	+	+	+	+	–	–	–	+	–	–	+	*Yr2, Yr5, Yr7, Yr9, Yr10, Yr18, Yr32*
8	Charmo	+	–	+	+	–	+	–	–	+	–	–	–	*Yr2, Yr7, Yr9, Yrvav, Yr18*
9	Hsad	–	–	+	+	–	–	–	–	–	+	+	+	*Yr7, Yr9, Yr24, Yr26, Yr32*
10	Sarah	+	–	+	+	–	+	–	–	–	+	+	–	*Yr2, Yr7, Yr9, Yrvav, Yr24, Yr26*
11	Fath 1	+	–	+	+	+	–	–	–	–	+	+	–	*Yr2, Yr7, Yr9, Yr10, Yr24, Yr26*
12	Rezan	+	–	–	+	+	–	–	–	–	+	+	–	*Yr2, Yr9, Yr10, Yr24, Yr26*
13	Maaroof	+	–	+	+	–	+	–	–	–	+	–	–	*Yr2, Yr7, Yr9, Yrvav, Yr24*
14	Bura	+	+	+	+	–	+	–	–	–	+	+	–	*Yr2, Yr5, Yr7, Yr9, Yrvav, Yr24, Yr26*
15	Bhuth 10	+	–	+	–	+	–	–	–	+	+	+	–	*Yr2, Yr7, Yr10, Yr18, Yr24, Yr26*
16	Faris 1	+	+	+	+	+	–	–	–	–	+	+	–	*Yr2, Yr5, Yr7, Yr9, Yr10, Yr24, Yr26*
17	Iba 99	+	–	+	+	–	–	–	–	–	+	+	+	*Yr2, Yr7, Yr9, Yr24, Yr26, Yr32*
18	Bhuth 158	+	–	+	+	+	–	–	–	–	–	+	–	*Yr2, Yr7, Yr9, Yr10, Yr26*
19	Babil 113	+	+	+	+	–	–	–	–	–	+	+	–	*Yr2, Yr5, Yr7, Yr9, Yr24, Yr26*
20	Tamuz 2	+	–	+	–	–	–	–	–	–	+	+	–	*Yr2, Yr7, Yr24, Yr26*
21	Bhuth 22	+	–	+	+	–	–	–	–	–	–	+	–	*Yr2, Yr7, Yr9, Yr26*
22	Al-Rashid	+	–	+	+	+	–	–	–	–	+	+	–	*Yr2, Yr7, Yr9, Yr10, Yr24, Yr26*
23	Baghdad 3	+	–	+	+	–	+	–	–	–	+	+	–	*Yr2, Yr7, Yr9, Yrvav, Yr24, Yr26*
24	Iba 95	+	–	+	+	–	–	–	–	–	+	+	–	*Yr2, Yr7, Yr9, Yr24, Yr26*
25	Adana	+	–	+	+	+	–	–	–	–	–	+	–	*Yr2, Yr7, Yr9, Yr10, Yr26*
26	Wafia	–	–	–	+	–	–	–	–	–	–	+	–	*Yr9, Yr26*
27	Baghdad 1	+	–	–	+	–	–	–	–	–	–	+	–	*Yr2, Yr9, Yr26*
28	Latifia	+	–	–	+	–	–	–	–	–	–	+	–	*Yr2, Yr9, Yr26*
29	Bengal	+	–	+	–	–	–	–	–	–	–	+	–	*Yr2, Yr7, Yr26*
30	Abu Ghraib	+	–	+	+	–	–	–	–	–	–	+	–	*Yr2, Yr7, Yr9, Yr26*
31	Uruk	+	–	–	+	–	–	–	–	–	–	+	–	*Yr2, Yr9, Yr26*
32	Cham 6	+	–	–	–	–	–	–	–	–	–	+	–	*Yr2, Yr26*
33	SaberBeg	+	–	–	–	–	–	–	–	–	–	+	–	*Yr2, Yr26*
34	Al-Nur	+	–	+	–	–	–	–	–	–	–	+	–	*Yr2, Yr7, Yr26*
35	Barcelona	+	–	+	+	–	–	+	–	+	–	–	–	*Yr2, Yr7, Yr9, Yr15, Yr18*
36	Tamuz 3	+	–	–	–	–	–	–	–	–	–	–	–	*Yr2*
37	Iraq	+	–	+	+	–	–	–	–	–	–	–	–	*Yr2, Yr7, Yr9*
38	Ashur	+	–	–	+	–	–	+	–	–	–	–	–	*Yr2, Yr9, Yr15*

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Table 3 to be continued

No.	Cultivar name	*wmc364 (Yr2)*	*Xgwm501 (Yr5)*	*Xgwm526 (Yr7)*	*Xgwm582 (Yr9)*	*Xpsp3000 (Yr10)*	*Xpsp3000 (Yrvav)*	*Xgwm273 (Yr15)*	*VENTRIUP/LN2 (Yr17)*	*Xgwm295 (Yr18)*	*Barc181 (Yr24)*	*Barc181 (Yr26)*	*Wmc198 (Yr32)*	Detected *Yr* gene
39	Al-Madaen	+	–	+	–	–	–	–	–	–	–	–	–	*Yr2, Yr7*
40	Al-Samawa	–	–	+	+	–	–	–	–	–	–	–	–	*Yr7, Yr9*
41	Kalar 1	+	–	–	+	–	–	–	+	–	–	–	–	*Yr2, Yr9, Yr17*
42	Kalar 2	+	–	+	–	–	–	–	+	–	–	–	–	*Yr2, Yr7, Yr17*
43	Iratom	–	–	+	–	–	–	–	–	–	–	–	–	*Yr7*
44	Rabiea	+	–	+	+	–	–	–	–	–	–	–	–	*Yr2, Yr7, Yr9*
45	Al-Eiz	–	–	+	+	–	–	–	–	–	–	–	–	*Yr7, Yr9*
46	Qandaharia	+	–	+	+	–	–	–	–	–	–	–	–	*Yr2, Yr7, Yr9*

+ and – indicate the presence/absence of the corresponding *Yr* genes in the tested cultivars

2022). This outcome is consistent with some other studies that confirm the prevalence of Yr2 in a variety of cultivars (Rahmatov et al. 2019; Rani et al. 2019). *Yr5* was found in six cultivars (Al-Wand, Sulaimani 2, Baraka, Bura, Faris 1, and Babil 113) using the SSR marker *Xgwm501* (Figure 2 and Table 3). The amplified DNA segment of 166–172 bp in the gel electrophoresis and documentation verified the presence of *Yr5*. Since *Yr5* is present in few wheat cultivars and breeding lines globally, these results agree with some other studies (Wu et al. 2016; Zheng et al. 2017). *Yr5* confers resistance against most *Pst* races worldwide, with the exception of those from India and Australia (Rani et al. 2019). It has been used in breeding programmes and transferred into wheat cultivars (Pourkhorshid et al. 2014). *Yr5* has been effective in Iran, Iraq, and other countries, making it a strong conductor for wheat breeding programs (Al-Maaroof 2022).

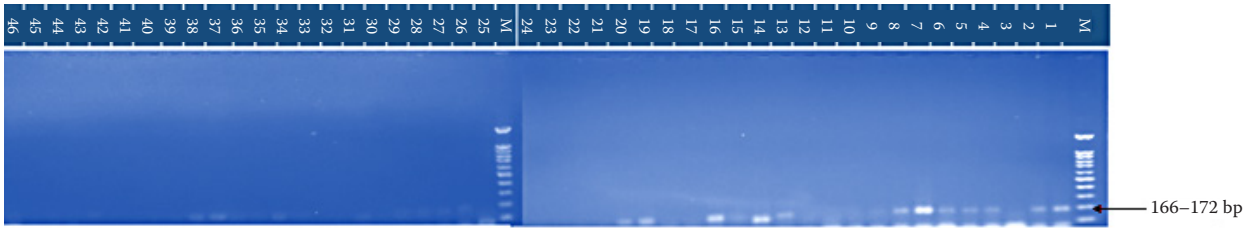

Figure 2. SSR amplification profile for primer *Xgwm501* (*Yr5*) on 46 wheat cultivars
M – 100 bp DNA ladder; the numerical designation of the genotypes is shown in Table 3

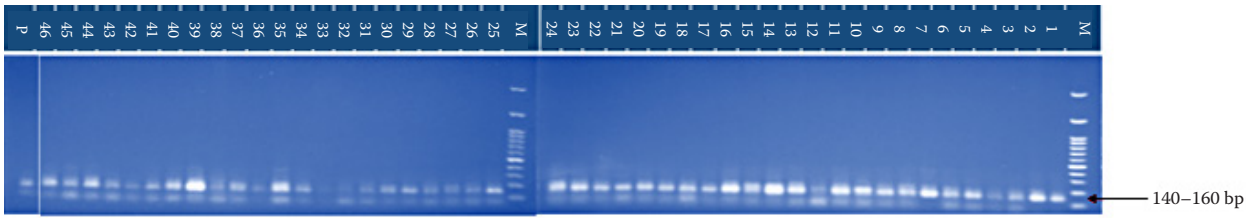

Figure 3. SSR amplification profile for primer *Xgwm526* (*Yr7*) on 46 wheat cultivars
M – 100 bp DNA ladder; P – positive control; the numerical designation of the genotypes is shown in Table 3

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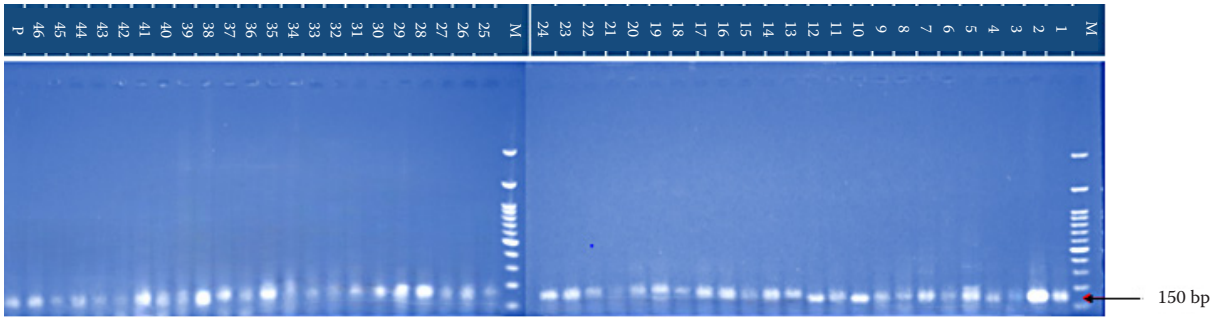

Figure 4. SSR amplification profile for primer *Xgwm582* (*Yr9*) on 46 wheat cultivars
M – 100 bp DNA ladder; P – positive control; the numerical designation of the genotypes is shown in Table 3

The microsatellite marker *Xgwm526* with PCR product band size of 140–160 bp explored the presence of *Yr7* in 76.09% of the cultivars. This was further verified by using YR7/6 × AOC genotypes carrying *Yr7* gene as positive control (Figure 3 and Table 3). This result agrees with Fayadh and Awad (2017), who referred earlier to the presence of *Yr7* in a variety of wheat cultivars. Recently, *Yr7* with several resistance genes have been rendered ineffective by the novel widespread *Pst* races (Rani et al. 2019). The majority of the common races in Iraq are virulent on *Yr7* (Al-Maaroof 2022).

The APR gene *Yr9* on chromosome 1BS is derived from *Secale cereale* (Zeng et al. 2014). *Xgwm582* amplifies a 150 bp PCR product indicating the presence of *Yr9* in most wheat cultivars except Hawler 2, Alaa, Bhuth10, Tamuz 2, Bengal, Cham 6, SaberBeg, Al-Nur, Tamuz 3, Al-Madaen, Kalar 2 and Iratom (Figure 4, Table 3). YR9/6 × AOC genotypes carrying *Yr9* are used as a positive control. The presence of *Yr9* in 34 cultivars in this study agrees with previous studies that noted *Yr9*'s presence in a variety of Iraqi cultivars (Fayadh & Awad 2017). In 1998, YR outbreaks in most of Iraq's wheat fields resulted in a notable 33% grain reduction in Maxipak and 45.9% in SaberBeg (Al-Maaroof et al. 2003)

Yr10 and *Yrvav*, the two alternative alleles of *Yr10*, were linked to the *Xpsp3000* marker (Bariana et al. 2002). The results of this study show that19.6% of the tested cultivars including Hawler 4, Baraka, Fath 1, Rezan, Bhuth 10, Faris 1, Bhuth 158, Al-Rashid and Adana produced 260bp fragments, indicating the presence of *Yr10*, while only six cultivars Al-Wand, Charmo, Sarah, Maaroof, Bura and Baghdad 3 amplify 285bp band associated with *Yrvav* allele (Figure 5, Table 3). Meanwhile, the rest cultivars lack both genes. Most of the earlier research discussed the small number of cultivars that have *Yr10* (Tahir et al. 2020; Kokhmetova et al. 2021).

Yr15 was found in only 8.7% of the cultivars, including Al-Wand, Sulaimani 2, Barcelona, and Ashur (Figure 6, Table 3). The SSR marker *Xgwm273* was used to identify *Yr15* in the cultivars; PCR results amplified 156–200 bp. This result agrees with the findings of other studies (Wu et al. 2016). A few significant *Yr* genes, such as *Yr5* and *Yr15*, are still effective against the post-2000 *Pst* virulent races (Dang et al. 2022).

The presence of *Yr17* in wheat cultivars using STS (*VENTRIUP/LN2*) marker, revealed that only Kalar 1 and Kalar 2 amplified a 259 bp fragment specific for *Yr17*, indicating the presence of *Yr17*

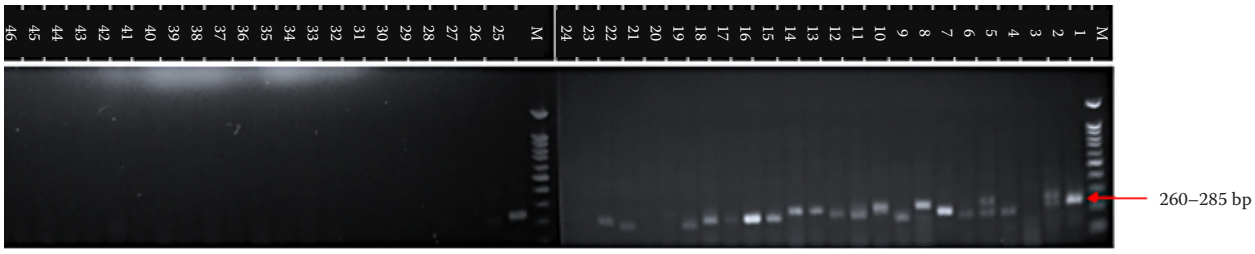

Figure 5. SSR amplification profile for primer *Xpsp3000* (*Yr10/Yrvav*) on 46 wheat cultivars
M – 100 bp DNA ladder; the numerical designation of the genotypes is shown in Table 3

https://doi.org/10.17221/39/2024-CJGPB

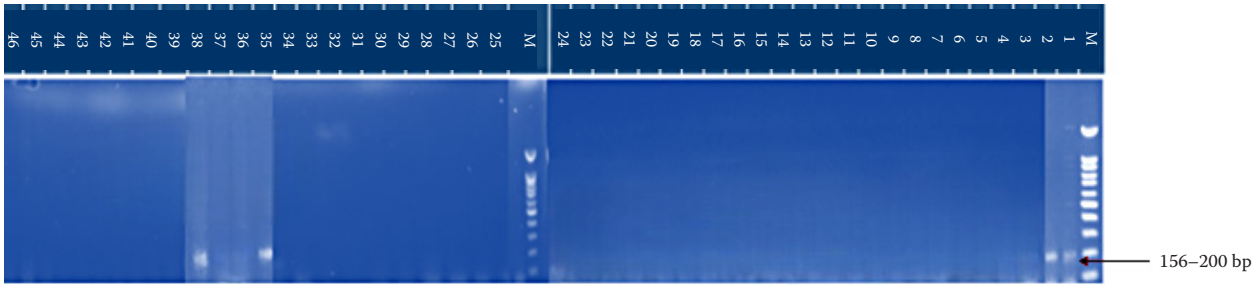

Figure 6. SSR amplification profile for primer *Xgwm273* (*Yr15*) on 46 wheat cultivars
M – 100 bp DNA ladder; the numerical designation of the genotypes is shown in Table 3

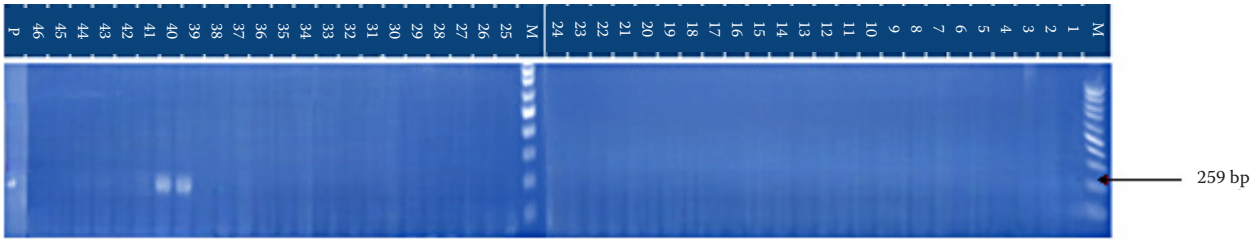

Figure 7. STS amplification profile for primer *VENTRIUP/LN2* (*Yr17*) on 46 wheat cultivars
M – 100 bp DNA ladder; P – positive control; the numerical designation of the genotypes is shown in Table 3

in these cultivars. The positive control in this case was YR17/6 × AOC differential (Figure 7, Table 3). This result was consistent with the fact that a limited number of wheat cultivars possess *Yr10, Yr15, Yr17, Yr18, Yr26* and *Yr36* (Zheng et al. 2017; Rahmatov et al. 2019; Kokhmetova et al. 2021; Tahir et al. 2020). *Yr17* was also rendered with some new *Pst* races (Rani et al. 2019).

The SSR marker *Xgwm295* was used to test the presence of *Yr18*. Of the 46 tested cultivars in this study, only four (8.7%) displayed amplified 160–250 bp fragments in Baraka, Charmo, Bhuth10 and Barcelona indicating the presence of this gene (Figure 8, Table 3). This outcome validates the findings of another researcher. In fact, few wheat cultivars defined the existence of *Yr18* (Wu et al. 2016; Intikhab et al. 2021; Zahid et al. 2022). The APR granted by *Yr18* was used in numerous breeding efforts across the globe and has been durable for over 50 years (Deng et al. 2022). Many resistance genes including *Yr18*, were defeated as a result of the quick emergence of new, virulent *Pst* races (Rani et al. 2019). Virulence against *Yr18* was formerly reported in Iraq (Al-Maaroof et al. 2003, 2022).

It has been proposed that *Yr26, Yr24* and *YrCH42* are related genes since they generate similar IT against 26 *Pst* isolates and are active against many *Pst* races (Deng et al. 2022). Using *Xbarc181* marker, The PCR produces two bands of distinct sizes that are easily defined on an agarose gel: a 180 bp fragment specific

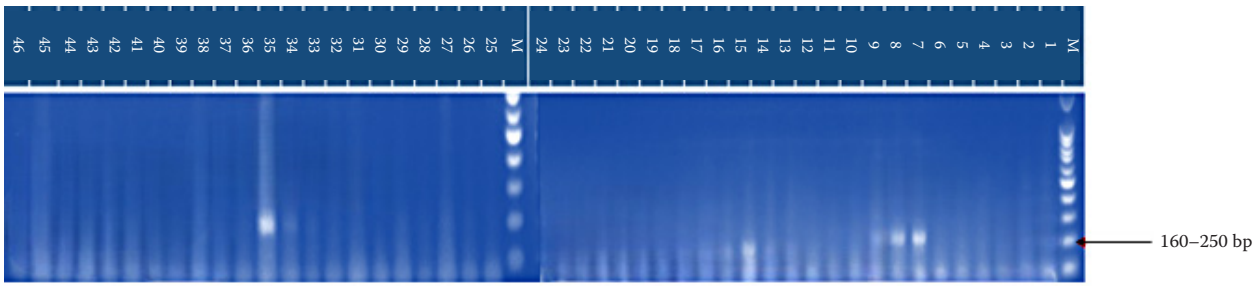

Figure 8. SSR amplification profile for primer *Xgwm295* (*Yr18*) on 46 wheat cultivars
M – 100 bp DNA ladder; the numerical designation of the genotypes is shown in Table 3

https://doi.org/10.17221/39/2024-CJGPB

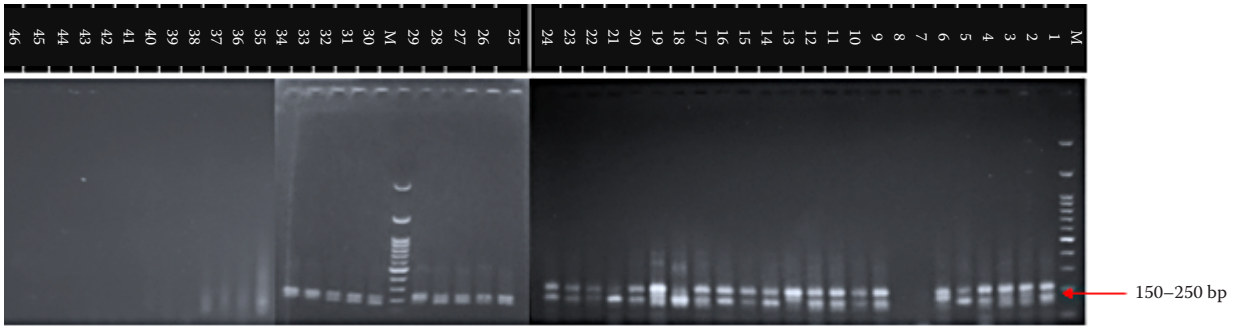

Figure 9. SSR amplification profile for primer *Barc181* (*Yr24/26*) on 46 wheat cultivars
M – 100 bp DNA ladder; the numerical designation of the genotypes is shown in Table 3

to the *Yr26* allele and a 220 bp fragment specific to the *Yr24* allele, respectively. In the current, 180 bp alleles related to *Yr26* were produced by 12 cultivars (26.09%), including Bhuth158, Bhuth 22, Adana, Wafia, Baghdad 3, Latifia, Bengal, Abu Ghraib, Uruk, Cham 6, SaberBeg and Al-Nur. while only Maaroof (2.17%) amplified the 220bp specific allele linked with *Yr24*. Nineteen genotypes (41.3%) including Al-Wand, Sulaimani 2, Hawler 2, Hawler 4, Azmar, Alaa, Hsad, Sarah, Fath 1, Rezan, Bura, Bhuth 10, Fars 1, Iba 99, Babil 113, Tamuz 2, Al-Rashid, Baghdad 3, Iba 95 amplified both alleles. Results obtained by combining both markers show that 32 cultivars (69.6%) have *Yr24/Yr26* (Figure 9, Table 3).

Only five cultivars (Al-Wand, Sulaimani 2, Baraka, Hsad and Iba 99) exhibited the 159–160 bp band amplified by the SSR marker *wmc198,* suggesting the presence of *Yr32* (Figure 10, Table 3). This result agrees with other research findings that refer to the *Yr32* presence in small number of cultivars (Rani et al. 2019). Effective resistance to YR is still present in many APR genes, including *Yr32* (Tahir et al. 2020).

Table 3 shows that Al-Wand has the most *Yr* genes (*Yr2*, *Yr5*, *Yr7*, *Yr9*, *Yrvav*, *Yr15*, *Yr24*, *Yr26* and *Yr32*), while Sulaimani 2 has 8 *Yr* genes (*Yr2*, *Yr5*, *Yr7*, *Yr9*, *Yr15*, *Yr24*, *Yr26* and *Yr32*). Bura, Fars1 and Baraka have 7 *Yr* genes; while, 7 cultivars- Sarah, Fath1, Bhuth 10, Iba 99, Babil 113, Al-Rashid and Baghdad 3 have 6 *Yr* genes, 10 cultivars- Hawler 4, Azmar, Hsad, Charmo, Rezan, Maaroof, Bhuth 158, Iba 95, Adana and Barcelona have 5 *Yr* genes. Four cultivars with 4 *Yr* genes include Hawler 2, Tamuz 2, Bhuth 22 and Abu Ghraib. Moreover, 12 cultivars- Alaa, Baghdad 1, Latifia, Bengal, Uruk, Al-Nur, Iraq, Ashur, Kalar 1, Kalar 2, Rabiea and SaberBeg contain 3 *Yr* genes, and 6 cultivars- Wafia, Cham 6, Qandaharia, Al-Madaen, Al-Samawa and Al-Eiz contain just 2 *Yr* genes. Iratom and Tamuz 3 share a single *Yr* gene.

Pst resistance sources can be pyramided into commercial cultivars using cultivars with several *Yr* genes (Zeng et al. 2014). *Yr2*, however, was the most often found SR gene in this study (87.0%); several *Pst* races in Iraq are virulent to *Yr2* (Loladze 2006). Moreover, the new emerging *Pst* races have rendered *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr18*, *Yr19*, *Yr21*, *Yr22*, *Yr23*, *Yr25*, *Yr27* and *YrA* ineffective (Rani et al. 2019; Al-Maaroof 2022; Al-Maaroof et al. 2022). The second and third most common *Yr* genes are *Yr7* and *Yr9* which together account for 76.1% and 73.9% of all *Yr* genes. Since many *Pst* races carry virulence on both genes, they are not relevant for Iraqi breeding programs. Followed by *Yr26* (67.4%), *Yr24* (43.5%), *Yr10* (19.6%), *Yr5* (17.4%), *Yrvav* (13.0%), *Yr32* (10.9),

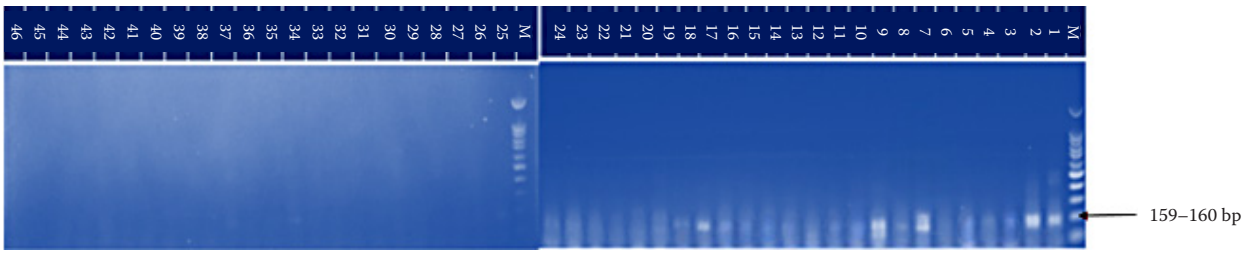

Figure 10. SSR amplification profile for primer *Wmc198* (*Yr32*) on 46 wheat cultivars
M – 100 bp DNA ladder; the numerical designation of the genotypes is shown in Table 3

https://doi.org/10.17221/39/2024-CJGPB

Yr15, *Yr18* (8.7%) and *Yr17* (4.4%). Wheat breeders can use the study results to use marker assisted selection to pyramid *Yr* resistance genes in future wheat cultivars.

Field base response of wheat cultivars to YR is positively correlated with molecular diagnosis results in this study. Al-Wand, Rezan and Sarah showed the best levels of disease resistance with the lowest DS and CI of all the tested cultivars. These cultivars have several *Yr* genes and outperformed all other cultivars in both seasons. There is 9 *Yr* genes in Al-Wand, 6 *Yr* genes in Sarah (*Yr2*, *Yr7*, *Yr9*, *Yrvav*, *Yr24* and *Yr26*), and 5 *Yr* genes in Rezan (*Yr2*, *Yr9*, *Yr10*, *Yr24* and *Yr26*). On the other hand, Kalar1 was found to have 3 *Yr* genes (*Yr2*, *Yr7* and *Yr17*) that showed R response in the field. However, Kalar 1 might harbour other unknown effective resistant genes. Moreover, some Yr genes that control YR are often linked to genes that control resistance to other rusts like stem and leaf rust, which are important in many cultivation areas in Iraq, such as *Lr37-Yr17-Sr38* complex (Iqbal et al. 2016; Nazari et al. 2021; Al-Maaroof 2022). Future studies should, therefore, identify the source of this cultivar's genetic resistance.

Certain wheat cultivars explored HS with high levels of DS and IT in the field during both seasons. Qandaharia (*Yr2*, *Yr7*, and *Yr9*), Al-Madaen (*Yr2* and *Yr7*), SaberBeg and Cham 6 (*Yr2* and *Yr26*), Wafia (*Yr9* and *Yr26*), Latifia, Bengal, and Uruk (*Yr2*, *Yr7*, and *Yr26*), Tamuz 2 (*Yr2*, *Yr7*, *Yr24*, and *Yr26*), and Bhuth 22 (*Yr2*, *Yr7*, *Yr9*, and *Yr26*) are among the cultivars that possess a number of ineffective *Yr* genes. As *PstS2* and *PstS2v27* races are common in Sulaimania, most of these genes are ineffective against them (Al-Maaroof et al. 2015, 2022). Other cultivars have some effective *Yr* genes but they are also susceptible in the field. For example, Hsad has (*Yr7*, *Yr9*, *Yr24*, *Yr26*, and *Yr32*), Tamuz 3 has (*Yr2* and *Yr5*), Bhuth 158 has (*Yr2*, *Yr7*, *Yr9*, *Yr10*, and *Yr26*), Ashur has (*Yr2*, *Yr9*, and *Yr15*), Baraka has (*Yr2*, *Yr5*, *Yr7*, *Yr9*, *Yr10*, *Yr18*, and *Yr32*), Bhuth 10 has (*Yr2*, *Yr7*, *Yr10*, *Yr18*, *Yr24,* and *Yr26*), Fath 1 has (*Yr2*, *Yr7*, *Yr9*, *Yr10*, *Yr24*, and *Yr26*), and Iba 99 has (*Yr2*, *Yr7*, *Yr9*, *Yr24*, *Yr26,* and *Yr32*).

CONCLUSION

YR is the major biotic stress for wheat production in Iraq. The majority of wheat cultivars are susceptible to YR in Sulaimania. However, Al-Wand, Kalar 1, Rezan, and Sarah are R and Al-Rashid, Charmo, Faris 1, Maaroof, Rabiea, and Iratom are MR. This could be the outcome of the limited genetic sources used by the breeders to improve YR resistance in Iraq. The majority of *Yr* genes have been found ineffective against *Pst* races *Yr5* and *Yr15* are effective against the dominant *Pst* races in Sulaimania; this suggests that some of these *Yr* genes may be defeated by the new *Pst* races, particularly in the 2023 epidemic, or other cultivars may have additional unidentified ineffective *Yr* genes. The reason for the limited success of introducing individual SR genes into commercial cultivars is that they don't work well against the newly emerging virulent *Pst* races. Pyramiding 2 or more SR genes and/or using multiple APR genes is recommended for durable resistance (Bariana et al. 2010). Al-Wand and Sulaimani 2 have many SR and APR *Yr* genes, introducing a number of additional genes in these cultivars makes them more resistant to YR. The genetic characterisation of different resistance sources is essential to disseminating their combinations in commercial cultivars. Race-specific resistance genes that trigger a hypersensitive response to stop *Pst* growth are typically responsible for YR control. Durable resistance in adapted wheat cultivars requires the availability of several sources of resistance to pyramiding (Liu et al. 2020). Therefore, the current study is crucial to identify several *Yr* genes in Iraqi wheat cultivars in order to validate the accumulation of several resistance genes in a single cultivar. This will protect the new cultivars from virulent *Pst* races, cost-effective, durable and environmentally safe. To improve YR management, more efforts have to be made to diversify the genetic resources and keep an eye on the pathogen population.

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