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Race specificity of stripe rust resistance in relation to susceptibility of Egyptian wheat cultivars

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Abstract

Race - specific resistance of stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) in relation to susceptibility of Egyptian wheat cultivars were investigated during two growing seasons 2017/18 and 2018/19 in Egypt. High levels of adult plant susceptibility were recorded in the second season in comparison with that of the first season. Out of 20 cultivars tested, susceptibility (S) up to 80 S was recorded with only 8 cultivars and 4 resistance genes (*Yr*) in the first season. However, it was recorded with 14 cultivars (reached 90 S) and 7 *Yr* genes (up to 80 S) in the second one. The highest susceptibility was recorded with Sids-12 and Gemmeiza-11 (90 S), followed by Misr-1 (80 S), Misr-2 (70 S), Giza-163 (60 S), *Yr7* and *Yr9* (80 S). Cultivars, Misr-1, Misr-2, Gemmeiza-5, Gemmeiza-12, Giza-167, and Shandweel-1, exhibiting moderate resistance (MR) in the first season, lost their resistance in the second one. Nine *Yr* genes *Yr2*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr25*, *Yr27*, *Yr32* and *YrSp* were postulated in cultivars through multipathotyping with the prevailing *Pst* races in Egypt, 64E0, 0E16, 66E0, 4E130, 2E0, 2E16, 4E0, 6E4, 70E4, *PstS2*, *PstS3* and Triticale aggressive. Only *Yr9* was present in 7 susceptible cultivars, Sids-13, Gemmeiza-7, Gemmeiza-9, Gemmeiza-10, Gemmeiza11, Gemmeiza-12, and Shandweel-1. A molecular assay using the STS marker *iag95* validated the presence of *Yr9* in the last-mentioned cultivars. Cultivars may have become susceptible due to the virulence to *Yr9* gene, which should be restricted to use alone in wheat production.

Keywords: *Triticum aestivum*, *Puccinia striiformis*, Resistance genes, Cultivar susceptibility, Race specificity.

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Introduction

Stripe (yellow) rust disease caused by *Puccinia striiformis* West. f. sp. *tritici* (*Pst*) is a major threat to wheat production worldwide (Chen *et al*., 2014; Kumar *et al*., 2016), particularly in temperate, humid and cooler wheat-growing regions of the world (Chen, 2005). Up to 88% of the world's wheat cultivars had become susceptible since 1960 and that annual loss amounted to 5.47 million tonnes (Beddow *et al*., 2015). Grain yield losses of 10 to 70% have been recorded in most wheat-producing areas of the world, which varies with the cultivar susceptibility, initial infection, disease period and development rate (Chen 2005). In Egypt, the disease affected most of the wheat cultivars, since major epiphytotic has been recorded once in every decade since the Sixties, causing grain yield loss ranged from 14% to 26% in the Nile Delta region and 10% loss countrywide (El-Daoudi *et al*., 1996). Draz *et al*. (2018) reported grain weight loss of up to 23.12% (Giza-160) in Egypt. The

most economical and eco-friendly approach to control the disease is the use of resistant wheat varieties (Pink, 2002; Chen, 2013; Yang *et al*., 2019). Breeding programs for disease resistance mainly aim to provide host materials with combinations of effective resistance genes concerning the prevalent population of the pathogen. Usually, selection breeding for resistance is solely based on field evaluations, while the precise constitution of the gene involved in the resistant germplasm is little known.

It is a challenge to avoid stripe rust epidemics in Egypt due to numerous wheat cultivars without genetic information that have been released and cultivated countrywide. Those cultivars may consist of the same gene or combined genes for resistance which may lead up to pressure the selection for corresponding virulence races. Therefore, identifying the resistance genes in wheat cultivars is essential for the development of cultivars with effective genes to control the disease. To date, 80 resistance genes of stripe rust (*Yr*) have been permanently named in wheat (McIntosh *et al*., 2017; Feng *et al*., 2018; Nsabiyera *et al*., 2018; Long *et al*., 2019), out of them, 67 *Yr* genes have been temporarily designated, including seedling resistance (all-stage resistance) and adult plant resistance (APR) (Wang and Chen, 2017). Among these, *Yr11*, *Yr12*, *Yr13*, *Yr14*, *Yr16*, *Yr18*, *Yr29*, *Yr30*, *Yr34*, *Yr36*, *Yr39*, *Yr46*, *Yr48*, *Yr52* and *Yr67* confer adult plant resistance, whereas the others confer all-stage resistance (race-specific resistance) *e.g Yr2*, *Yr4*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr19*, *Yr25*, *Yr26*, *Yr27*, *Yr28*, *Yr35*, *Yr36*, *Yr37*, *Yr38*, *Yr40*, *Yr42 Yr53*, *Yr61*, *Yr65* and *Yr69* (Chen, 2005; Zheng *et al*., 2017). Of these, only *Yr5* and *Yr15*, *Yr53*, *Yr61*, *Yr65*, and *Yr69* still confer resistance to most *Pst* races and can be used in breeding for disease resistance (Xu *et al*., 2013; Zeng *et al*., 2014; Zhou *et al*., 2014; Yang *et al*., 2016; Shahin, 2017). Even though these *Yr* genes have been detected in various wheat varieties, but their efficacy against the diverse *Pst* pathotypes is limited due to the race specificity of all-stage resistance. By contrast, APR is generally considered long-lasting, but its known genes represent a minority (Chen, 2005; Ellis *et al*., 2014; Kankwatsa *et al*., 2017).

Epidemiological concerns suggest that it should deploy different resistance genes in wheat cultivars for controlling wheat stripe rust in the affected regions (Wan *et al*., 2007; Zeng, 2014). Therefore, a further survey of cultivar susceptibility along with race specificity of seedling resistance genes in local wheat cultivars could provide information concerning the epidemiology and evolution of different populations of the pathogen and its responsibility in the cultivar susceptibility. To apply this approach, it is important to know which resistance genes are already existed in cultivars and being used in breeding programs in the epidemiological regions.

Screening of resistance genes through multipathotypic inoculation test (known as gene postulation) with a set of virulent (*Av*)/ virulent (*v*) races of the pathogen is an effective approach to determine which genes are existed in wheat cultivars (Browder and Eversmeyer, 1980; Statler, 1984; Wang *et al*., 2009), which it has been used since the discovery of the gene-for-gene concept by Flor (1959). The approach relies on the interaction between the gene of the host lines and *Av*/*v* gene of pathogen races to determine the probable resistance genes in wheat cultivars. Several researchers have employed gene postulation for identifying *Yr* genes in a group of wheat genotypes (Sharma *et al*., 1995; Hovmøller, 2007; Xia *et al*., 2007; Dawit *et al*., 2012; El Amil *et al*., 2019). Also, the development of molecular markers has led to efficient methods of plant breeding for disease resistance, which is a reliable method in identifying resistance genes in wheat. DNA markers, such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple-sequence-repeat (SSR), and sequence-tagged site (STS) have been widely used in conjugation with pedigree information and gene postulation to identify *Yr* resistance genes in wheat cultivars and advanced breeding lines (Wang *et al*., 2009; Zhou *et al*., 2014; Zeng *et al*., 2014; Gebreslasie *et al*., 2020). In the present study, we report race-specific resistance genes (*Yr*) responsible for the susceptibility of Egyptian wheat cultivars using the gene-for-gene theory and molecular marker to verify the *Yr* gene(s) postulated in cultivars.

Materials and methods

Plant materials

Twenty Egyptian wheat cultivars (Table 1) were tested for adult plant susceptibility to stripe rust and the presence/absence of stripe rust race-specific resistance genes. In this study, nine race-specific resistance genes *Yr2*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr25*, and *Yr27*, corresponding to virulence spectra of the contemporary Egyptian population of *P. striiformis* f. sp. *tritici* during

the respective period (2016-2018) were selected. The tested genes in Kalyasona (*Yr2*), Hybrid-46 (*Yr4*), TP1295 (*Yr25*), near-isogenic lines (NILs) in Avocet (AOC) with *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr27*, were kindly provided by CIMMIYT, Mexico.

Pst races

Twelve *Pst* races, 64E0, 0E16, 66E0, 4E130, 2E0, 2E16, 4E0, 6E4, 70E4, *PstS2*, *PstS3* and Triticale aggressive reported in our previous study during 2016-2018 in Egypt (Draz *et al*., 2019; Draz, 2019) were used in this study. The 12 *Pst* races have virulence spectra to the tested *Yr* genes as shown in Table (2).

Evaluation of adult plant susceptibility

In the open field, the adult plant susceptibility of the tested wheat cultivars and *Yr* genes (Table 1) to stripe rust was evaluated under natural infection. The experiments were carried out during two growing seasons (2017/18 and 2018/19) at the Experimental Farms of Sakha Agricultural Research Station, Agricultural Research Center, Egypt. A triple experiment was performed in a complete randomized block. The sowing date was in mid-November. Seeds of the tested cultivars/lines were sown in 3 m long rows (3 rows/plot per cultivar) with 30 cm apart and 5 g seed per row. The experiment was surrounded by a 1.5 m 2 belt of the susceptible variety Morocco served as a spreader of natural infection and susceptible check. All cultural practices recommended for the wheat crop were applied. Adult plant susceptibility to wheat stripe rust was assessed at the early dough stage (Large 1954) when rust symptoms have fully developed in comparison with the susceptible check cultivar Morocco. Disease assessment was scored based on host responses as described in Table (3) according to Roelfs *et al*. (1992) along with rust severity expressed as percentage coverage of leaves with rust pustules following Cobb's scale modified by Peterson *et al*. (1948).

Table 1 Egyptian wheat cultivars used in this study and their pedigree

Cultivar	Pedigree
Misr-1	OASIS/SKAUZ//4*BCN/3/2*PASTOR.CMSSOYO1881T-050M-030Y-O3OM-030WGY-33M-0Y-0S
Misr-2	SKAUZ/BAV92.CMSS96M0361S-1M-010SY-010M-010SY-8M-0Y-0S
Misr-3	CMSS06Y00582T099TOPM-099Y-099ZTM-009Y-099M-10WGY-0B-0EGY
Sids-12	BUC//7C/ALD/5/MAYA74/ON//1160-147/3/BB/GLL/4/CHAT"S"/6/MAYA/VUL//CMH74A.630/4*SX
Sids-13	KAUZ "S"//TSI/SNB"S". ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP-0SD
Sakha-61	Inia–RL4220//7C/YR ‘‘S’’ CM15430-25-55-0S-0S
Sakha-93	Sakha92/TR810328S8871-1S-2S-1S-0S
Sakha-94	Opata/Rayon//Kauz CMBW9043180-OTOPM-3Y-010M-010M-010Y-10M-015Y-0Y-0AP-0S
Sakha-95	CMA01Y00158S-040POY-040M-030ZTM-040SY-26M-0Y-0SY-0S
Gemmeiza-5	Vee”S”/SWM6525GM.4017-1GM.7GM-3GM-0GM
Gemmeiza-7	CMH74A.630/SX//SER182/3/AGENT. GM4611-2GM-3GM-1GM-0GM
Gemmeiza-9	ALD"S"/HUAC"S"//CMH74A.630/SX. GM4583-5GM-1GM-0GM
Gemmeiza-10	Maya74‘‘S’’/on/1160-147/3/Bb/G11/4/chat‘‘S’’/5/crow‘‘S’’CG5820-3G-1G-2G-0G
Gemmeiza-11	BOW ‘‘S’’/KVZ ‘‘S’’//7C/SERI82/3/GIZA168/SKHA61
Gemmeiza-12	OTUS/3/SARA/THB//VEECMSS97Y00227S-5Y-010M-010Y-010M-2Y-1M-0Y-0GM
Giza-163	*T. aestivum*/Bon//Cno/7CCM33009-F-15M-4Y-2M-1M-1M-1Y0M
Giza-167	Au/UP301//G11/SX/Pew‘‘S’’/4/Mai‘‘S’’/May‘‘S’’//Pew‘‘S’’CM67245-C-1M-2Y-1M-7Y-1M-0Y
Giza-168	MRL/BUC//Seri.CM93046-8M-0Y-0M-2Y-0B
Giza-171	Sakha93/Gemmeiza9S.6-1GZ-4GZ-1GZ-2GZ-0S
Shandweel-1	SITE//MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC.CMSS93B00567S-72Y-010M-010Y-010M-0HTY

Multipathotypic test

To postulate stripe rust race-specific resistance genes (*Yr*) in wheat cultivars, the gene-for-gene concept for infection type data was applied according to the method of Browder and Eversmeyer (1980) and Statler (1984). In this test, multipathotyping was carried out to the tested wheat accessions through the inoculation with the 12 races of *P. striiformis* f.sp. *tritici* used in this study. In which, 8-day-old seedlings of wheat cultivars/lines were inoculated with urediniospores of each *Pst* race according to the method described by Stubbs (1988). The inoculated seedlings were misted with water and incubated in a dark dew chamber at 10°C for 24 h under high relative humidity (RH). After incubation, the inoculated seedlings were transferred to a greenhouse with conditions of 100% RH and 13±2°C under 16 h photoperiod with a light intensity of 100 µmol m^{-2} sec^{-} Experiments were performed in three replications in the greenhouse at the Wheat Disease Research Department, Sakha Agricultural Research Station of Egypt. Disease scoring was made 15-18 days after inoculation. First seedling leaf was considered for phenotyping on a 0-9 scale (McNeal *et al*., 1971), where infection types 0 to 6 refer to resistant and 7 to 9 susceptible.

Table 2 The prevailing races of *P. striif*ormis f. sp. *tritici* (*Pst*) in Egypt during 2016-2018 used in this study and their virulence spectra to the tested stripe rust race-specific genes (Yr)

***Pst* race**	**Virulence spectra**
0E16	*Yr8*
2E0	*Yr7*
4E0	*Yr2, Yr6*
64E0	*Yr4*
66E0	*Yr4, Yr7*
70E4	*Yr2, Yr4, Yr6, Yr7*
6E4	*Yr2, Yr6, Yr7, Yr25*
2E16	*Yr7, Yr8*
4E130	*Yr2, Yr6, Yr7, Yr25*
PstS2	*Yr2, Yr6, Yr7, Yr8, Yr9, Yr25, Yr27*
PstS3	*Yr2, Yr6, Yr7, Yr8, Yr25*
Triticale aggressive	*Yr2, Yr6, Yr7, Yr8, Yr10*

Table 3 Adult plant infection types of wheat stripe rust used in disease assessment

Infection type	**Host response**	**Disease symptoms**
0	Immune	No uredia or other symptoms of disease infection
R	Resistant	Uredia minute surrounded by distract necrotic area
MR	Moderately Resistant	Uredia small to medium usually in green islands surrounded by necrotic or chlorotic tissue
MS	Moderately Susceptible	Uredia medium in size with no necrosis but chlorosis may be present
S	Susceptible	Uredia large with no necrosis but chlorosis

Molecular marker assay

The stripe rust resistance genes postulated in the wheat cultivars based on infection types to *Pst* races were verified to be present based on gene-specific molecular markers. In this assay, the STS marker *iag95* with primer sequence fwd 5′ CTCTGTGGATAGTTACTTGATCGA 3′, rev 5′ CCTAGAACATGCATGGCTGTTACA 3′, was used to verify *Yr9* in cultivars (Mago *et al*., 2002). Genomic DNA was extracted from the fresh leaves using a Cetyl Trimethyl Ammonium Bromide (CTAB) protocol according to Doyle and Doyle (1987). Purification and quantification of DNA were determined via UV spectrophotometer measurement (Pharmacia, Biotech., Ultrospec 1000). The DNA samples were diluted for a final concentration of 10 ng/µl. Master Mix (Dream Taq Green PCR Master Mix (2X), Thermo Scientific), containing PCR reagents (dNTPs 0.4 mM each, 2X Taq DNA polymerase and 4 mM MgCl2) was used for PCR reaction. A total PCR reaction volume of 25 µl (12.5 µl of Master Mix, 0.8 µl of forward/reverse primer, 2 µl of DNA template, 9.7 µl sterile distilled water) was performed in a PCR condition (Techne, PROGENE Thermocycler) of 94°C 3 min (1 cycle), 94°C 30 s, 55°C 60 s, 72°C 70 s (30 cycles), 25 °C 60 s (1 cycle), according to the method described by Mago *et al*. (2002) with some modifications. PCR amplification products of 10 µl each sample were electrophoresed in 1.5% agarose gel stained with ethidium bromide at 100 V for about 20 min. A 100 bp DNA ladder H3 RTU, Nippon Genetics Europe GmbH, served in calibration. DNA bands were visualized using a UV-transilluminator (Herolab UVT 2020, Kurzwellig), then photographed.

Results

Adult plant susceptibility

Field data showed in Table 4 for adult plant reaction of wheat accessions against stripe rust revealed that the level of susceptibility in the 2018/19 growing season was higher than that observed in the 2017/18 growing season. Out of 20 cultivars and 9 wheat lines tested, susceptibility (S) was recorded with 14 Egyptian cultivars (Misr-1, Misr-2, Sids-12, Sids-13, Sakha-61, Gemmeiza-5, Gemmeiza-7, Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Gemmeiza-12, Giza-163, Giza-167, Shandweel-1) ranging between 30 S-90 S, and 7 wheat lines with *Yr* genes *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27* (20 MS-80 S) in 2018/19 growing season. However, it was recorded with only 8 cultivars, Sids-12, Sids-13, Sakha-61, Gemmeiza-7, Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Giza-163 and 4 lines with resistance genes *Yr6*, *Yr7*, *Yr9*, *Yr27* (10 S-80 S) in 2017/18 growing season. The highest level of susceptibility reaching 90 S was recorded with Egyptian cultivars, Sids-12 and Gemmeiza-11, followed by Misr-1 (80 S), Misr-2 (70 S), and Giza-163 (60 S) in 2018/19 growing season. However, it was up to 80 S in the 2017/18 growing season with only one cultivar Sids-12. Regarding wheat lines, *Yr7*, and *Yr9* showed the highest level of susceptibility (80 S), followed by *Yr6* (60 S) in the second season, while it was 80 S, 60 S, and 40 S, respectively in the previous season 2017/18. Wheat cultivars, Misr-1 (rated Tr MR), Misr-2 (Tr R), Gemmeiza-5, Gemmeiza-12 (10 MR), Giza-167 and Shandweel-1 (30 MR), exhibiting resistance in 2017/18 growing season, lost their resistance in the second growing season (2018/19), rating susceptibility of 80 S, 70 S, 30 S, 40 S, 30 S, 70 S, respectively. Likewise, the resistance of *Yr8* (5 MR), *Yr2* and *Yr25* (10 MR) observed in the first season, had been lost in the second season exhibiting moderate susceptible (20 MS) in *Yr2* and *Yr8*, and susceptible (30 S) in *Yr25*. Both *Yr4* and *Yr10* were still without symptoms of disease infection during both seasons. It was also observed that wheat cultivars, Misr-3, Sakha-93, Sakha-94, Sakha-95, Giza-168 and Giza-171 still be resistant during both growing seasons rating moderately resistance (MR).

Gene postulation and molecular marker

Data in Table 5 list the tested cultivars/lines with their seedling respective response spectra to 12 *Pst* races under greenhouse conditions. The results for each cultivar were interpreted based on the race-specific responses on the lines. Out of nine *Yr* genes tested, spectra appeared to represent the only *Yr9* to be postulated in eight Egyptian wheat cultivars, Sids-13, Sakha-94, Gemmeiza-7, Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Gemmeiza-12, and Shandweel-1. The presence of the *Yr9* gene in nine Egyptian wheat cultivars, Sids-13, Sakha94, Sakha-95, Gemmeiza-7, Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Gemmeiza-12, and Shandweel-1, was verified with a DNA fragment of 1100 bp (Fig.1).

Discussion

Wheat stripe rust epidemics usually occur due to new virulent races and favorable weather conditions. The constant change of *P. striiformis* f. sp. *tritici* populations is mainly due to the long-distance migration, high mutation, somatic recombination, and selection of the cultivars (Wan *et al*., 2004; Chen, 2005; Lei *et al*., 2017). Conventionally, breeders select wheat lines having strong all-stage resistance, which is controlled by a major gene and easy to be incorporated into new cultivars. Nevertheless, all-stage resistance is mostly race-specific, and new virulent races can easily overcome this. Widely growing of cultivars with race-specific resistance lead to new virulent races may become widespread and cause large-scale epidemics. In this case, we studied race-specificity of stripe rust resistance genes responsible for the susceptibility of Egyptian wheat cultivars during two growing seasons (2017/18 and 2018/19). Field evaluation revealed high levels of adult plant susceptibility in 2018/19 second season in comparison with that of 2017/18 first season. In 2018/19 season, it was recorded h 14 cultivars ranging between 30 S-90 S, and 7 race-specific resistance genes (*Yr*) rating 20 MS-80 S. However, it was recorded with only 8 cultivars and 4 *Yr* genes (10 S-80 S) in 2017/18 season.

Table 4 Adult plant reaction of wheat lines carrying race- specific resistance genes (*Yr*) and Egyptian wheat cultivars against stripe rust under field condition

Cultivar	Adult plant reaction	
	2017/18 season	2018/19 season
Kalyasona	10 MR	20 MS
Hybrid-46	0	0
***Yr6*/6*AOC**	40 S	60 S
***Yr7*/6*AOC**	80 S	80 S
***Yr8*/6*AOC**	5 MR	20 MS
***Yr9*/6*AOC**	60 S	80 S
***Yr10*/6*AOC**	0	0
TP1295	10 MR	30 S
***Yr27*/6*AOC**	10 S	30 S
Misr-1	Tr MR	80 S
Misr-2	Tr R	70 S
Misr-3	Tr MR	10 MR
Sids-12	80 S	90 S
Sids-13	30 MS	30 S
Sakha-61	10 S	30 S
Sakha-93	10 MR	20 MR
Sakha-94	20 MR	30 MR
Sakha-95	5 MR	10 MR
Gemmeiza-5	10 MR	30 S
Gemmeiza-7	30 S	40 S
Gemmeiza-9	30 S	40 S
Gemmeiza-10	40 S	50 S
Gemmeiza-11	30 S	90 S
Gemmeiza-12	10 MR	40 S
Giza-163	50 S	60 S
Giza-167	30 MR	30 S
Giza-168	10 MR	20 MR
Giza-171	10 MR	10 MR
Shandweel-1	30 MR	70 S
Morocco	80 S	90 S

Kalyasona: *Yr2*, Hybrid-46: *Yr4*, TP1295: *Yr25*, AOC: Avocet NILs with *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr27*, 0: No Symptoms, R: Resistant, MR:Moderately Resistant, MS: Moderately Susceptible, S: Susceptible

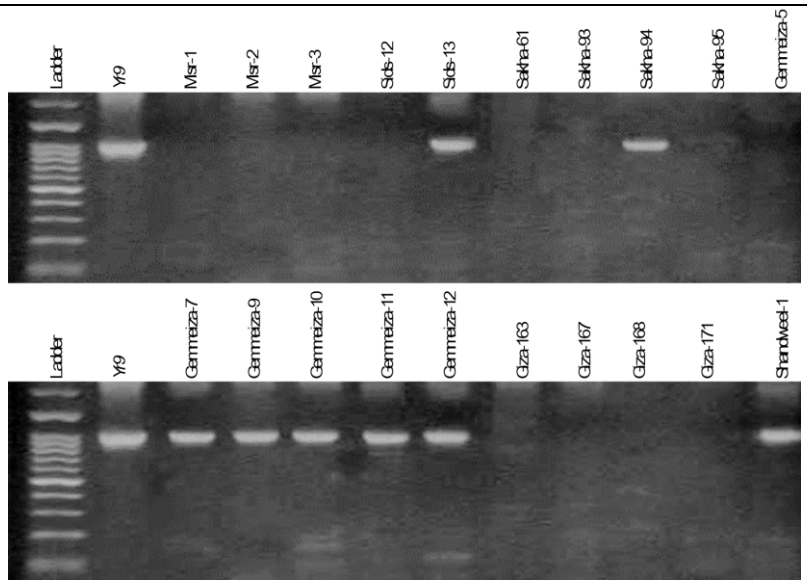

Fig.1 PCR of the STS marker iag-95 indicate the presence of races specific gene *Yr9* in eight Egyptian wheat cultivars with a DNA fragment of 1100-bp

Table 5 Seedling response of wheat lines carrying race-specific resistance genes (*Yr*) and Egyptian wheat cultivars to 12 races of *Puccinia striiformis* f. sp. *tritici* (*Pst*) and postulated genes.

Cultivar	*Pst* races/response												Postulated *Yr* gene
	0E16	2E0	4E0	64E0	66E0	70E4	6E4	2E16	4E130	*PstS2*	*PstS3*	Triticale aggressive	
Kalyasona	R	R	S	R	R	S	S	R	S	**S**	**S**	**S**	*Yr2*
Hybrid-46	R	R	R	S	S	S	R	R	R	R	R	R	*Yr4*
***Yr6*/6*AOC**	R	R	S	R	R	S	S	R	S	S	S	S	*Yr6*
***Yr7*/6*AOC**	R	S	R	R	S	S	S	S	S	S	S	S	*Yr7*
***Yr8*/6*AOC**	S	R	R	R	R	R	R	S	R	S	S	S	*Yr8*
***Yr9*/6*AOC**	R	R	R	R	R	R	R	R	R	S	R	R	*Yr9*
***Yr10*/6*AOC**	R	R	R	R	R	R	R	R	R	R	R	S	*Yr10*
TP1295	R	R	R	R	R	S	S	R	S	S	S	R	*Yr25*
***Yr27*/6*AOC**	R	R	R	R	R	R	R	R	R	S	R	R	*Yr27*
Misr-1	S	S	S	S	S	S	S	S	S	R	S	S	-
Misr-2	S	S	S	S	S	S	S	S	S	R	S	S	-
Misr-3	R	R	R	R	R	S	R	R	R	R	R	R	-
Sids-12	S	S	S	S	S	S	S	S	S	R	S	S	-
Sids-13	R	R	R	R	R	R	R	R	R	S	R	R	*Yr9*
Sakha-61	S	S	S	S	R	S	S	S	S	R	S	S	-
Sakha-93	S	S	S	S	S	S	S	S	S	R	S	S	-
Sakha-94	R	R	R	R	R	R	R	R	R	S	R	R	*Yr9*
Sakha-95	R	R	R	R	R	S	R	R	R	R	S	R	-
Gemmeiza-5	S	S	S	S	S	S	S	S	S	S	S	S	-
Gemmeiza-7	R	R	R	R	R	R	R	R	R	S	R	R	*Yr9*
Gemmeiza-9	R	R	R	R	R	R	R	R	R	S	R	R	*Yr9*
Gemmeiza-10	R	R	R	R	R	R	R	R	R	S	R	R	*Yr9*
Gemmeiza-11	R	R	R	R	R	R	R	R	R	S	R	R	*Yr9*
Gemmeiza-12	R	R	R	R	R	R	R	R	R	S	R	R	*Yr9*
Giza-163	S	S	S	S	S	S	S	S	S	R	S	S	-
Giza-167	S	S	S	S	S	S	S	S	S	R	S	S	-
Giza-168	S	S	S	R	R	S	S	S	S	R	S	S	-
Giza-171	S	S	S	R	R	S	S	S	S	R	S	S	-
Shandweel-1	R	R	R	R	R	R	R	R	R	S	R	R	*Yr9*
Morocco	S	S	S	S	S	S	S	S	S	S	S	S	-

The highest level of susceptibility reaching 90 S was recorded with cultivars, Sids-12 and Gemmeiza-11, followed by Misr-1 (80 S), Misr-2 (70 S), Giza-163 (60 S) and *Yr* genes *Yr7* and *Yr9* (80 S). Wheat cultivars, Misr-1, Misr-2, Gemmeiza-5, Gemmeiza-12, Giza-167, and Shandweel-1, rated moderately resistance (MR) in 2017/18 season, lost their resistance in the second season (2018/19) with susceptibility up to 80 S (Misr-1). Cultivars, Misr-3, Sakha-93, Sakha-94, Sakha-95, Giza-168 and Giza-171 rated moderately resistance (MR) during both seasons. These findings are in agreement with that of Shahin (2017) who reported that Egyptian cultivars such as Sids-12, Misr-2 and Sakha 61, previously known as resistant to stripe rust, have become susceptible. Also, virulence to several *Yr* genes e.g. *Yr2*, *Yr3a*, *Yr3b*, *Yr4a*, *Yr6*, *Yr6+2*, *Yr7*, *Yr8*, *Yr9*, *Yr9+2*, *Yr10*, *Yr17*, *Yr24*, *Yr27*, *Yr32*, *YrA*, *YrCV*, *YrSD* and *YrSU*, has been reported worldwide, including Egypt (Dawit *et al*., 2012; Shahin, 2017; Draz, 2019). Dawit *et al*. (2019) found that the differential lines carrying *Yr9*, *Yr8*, *Yr6* and *Yr7* had the highest rust severity of more than 80 S. Nazari and EL Amil (2013) reported that NILs *Yr6*, *Yr7*, and *Yr9* responded with high infection types. The *Yr9* was found among genes that are susceptible to 70-100% of sixty-one isolates of *P. striiformis* f. sp. *tritici* (Kumar *et al*., 2012). The resistance reduction of the most widely grown winter wheat cultivar "Eltan" in the US Pacific Northwest, was due to change of the *P. striiformis* f. sp. *tritici* population from avirulent to virulent, overcoming the race-specific all-stage resistance (Liu *et al*., 2019). The evolution of *Pst* races in Egypt during 2016-2018 has been reported with new virulent races which were used in the present study (Draz *et al*., 2019; Draz, 2019).

Specificity of host resistance expressed in distinct qualitative seedling reactions, through a challenge with a series of pathogen races, has often formed the basis for genetic analysis and gene postulation of both the host and the pathogen (Day, 1974; Johnson and Knott, 1992). We, therefore, postulated the race-specific resistance genes to stripe rust in 20 Egyptian wheat cultivars, and we tested them together with 12 *Pst* races at the seedling stage. This set of races represents the contemporary races in Egypt with different origins and virulence spectra to the tested *Yr* genes, thereby providing useful

information in relation to cultivar susceptibility. Out of nine *Yr* genes tested, *Yr9* was the commonly encountered gene alone in eight of the cultivars, Sids-13, Sakha 94, Gemmeiza-7, Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Gemmeiza-12 and Shandweel-1. The molecular assay using the STS marker *iag95* confirmed the presence of *Yr9* in the mentioned cultivars, corresponding to the results of multipathotyping test. This marker has been validated to detect *Yr9* gene in several studies (Mago *et al*., 2002; Rahmatov *et al*., 2019). El Amil *et al* (2019) performed multipathotype test on a collection of wheat elite lines, commercial varieties and landraces from Lebanon and Syria with 11 *Pst* pathotypes corresponding to *Yr1*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr9*, *Yr17*, *Yr25*, *Yr27*, *Yr32* and found that all of them except *Yr32* were postulated in wheat accessions. Several studies have reported numerous stripe rust race-specific genes *e.g. Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr5*, *Yr6*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr25*, *Yr26*, *Yr27*, *Yr32*, *YrSel*, *YrSD*, *YrA* and *YrSu*, in different collections of wheat cultivars and lines based on gene postulation and/or in conjugation with molecular markers (Sharma *et al*., 1995; Hovmøller, 2007; Xia *et al*., 2007, Wang *et al*., 2009; Zeng *et al*., 2014; Gebreslasie *et al*., 2020). In the current study, we are in the process of screening Egyptian wheat cultivars to identify ineffective genes that have race specificity to the prevailing *Pst* population in Egypt during 2016-2018. The evaluation of Egyptian wheat cultivars to stripe rust was carried out during 2017-2019 and the susceptibility was recorded with most cultivars tested. Therefore, we anticipate that *Yr* race-specific genes, tested to be present in cultivars, may be responsible for the cultivar susceptibility. Gene postulation based on response patterns of cultivars to the prevailing *Pst* population in Egypt revealed the presence of the *Yr9* gene in the majority of cultivars. We confirmed the presence of the *Yr9* gene in cultivars through the reliable marker iag95. Out of eight cultivars, Sids-13, Sakha-94, Gemmeiza-7, Gemmeiza-9, Gemmeiza-10, Gemmeiza11, Gemmeiza-12, and Shandweel-1, that confirmed to possess *Yr9* gene with a DNA fragment of 1100-bp, seven cultivars were susceptible to stripe rust except for cultivar Sakha-94 that exhibited moderate resistance. However, five cultivars Misr-3, Sakha-93, Sakha-95, Giza-168 and Giza-171 have moderate resistance to stripe rust during both seasons, but results indicated that they did not possess *Yr9*. These findings reveal that high susceptibility of Egyptian cultivars may be attributed to the virulence to *Yr9* gene which was found to be present in susceptible cultivars and absent in moderate resistant cultivars. The common ancestry of *Pst* population in Egypt was reported to be belonging to three races, including *PstS2* (Draz 2019). Our findings revealed that the *PstS2* virulence to the gene *Yr9* as well as the susceptible Egyptian cultivars was recorded.

The exception of cultivar Sakha-94 being moderately resistant to stripe rust and possessing *Yr9* may be attributed to suppression by a gene or genes in the cultivar Sakha-94. The gene in the heterozygous condition is suppressed by a gene or genes in wheat genome (Chen and Line, 1993). Shahin *et al*. (2018) reported the presence of adult plant resistance (APR) gene *Yr18* in Egyptian cultivar Sakha-94. Therefore, *Yr9* gene present in Sakha-94 may be suppressed due to *Yr18* gene that conferred partial resistance to the cultivar during both seasons. The *PstS2* race was first detected in 1980, originated from East Africa remains an important, globally prevalent race (Walter *et al.*, 2016; Ali *et al*., 2017). It caused severe losses in West and Central Asia in 2003, at a time that the breakdown of the resistance conferred by the widely deployed *Yr9* resistance gene was reported in the region (Singh *et al.*, 2004). The pathotyping of 214 samples collected from the Western Mediterranean region (Portugal, Spain, Southern France, Italy, Morocco, Algeria, Tunisia) and 54 samples from the eastern Mediterranean region (Cyprus, Turkey, Iran, Lebanon) identified 12 pathotypes during 2005–2006, including *PstS2* (Bahri *et al.*, 2009). The *Pst* population in Syria and Lebanon in 2010–2011 was dominated by the *PstS2* lineage (El Amil *et al*., 2020). Thus, this suggests that the Middle East, with its high degree of *Pst* population diversity, is a hotspot for the emergence of new *Pst* races. The aggressive, high temperature-adapted race *PstS2* virulent to *Yr9*, was detected between 2000 and 2004 in several European countries but at low frequencies (Hovmoller *et al*., 2008; de Vallavieille-Pope *et al*., 2012). The emergence of *PstS2* race led the stripe rust pathogen to spread to warmer regions and has become established in Western Australia, New Zealand, Southern and North Africa and the Southern United States (Milus *et al*. 2006; Wellings, 2011; Chen *et al*., 2014; Walter *et al*., 2016). Based on our findings, race-specific resistance gene *Yr9* present in the majority of Egyptian cultivars does not provide protection against stripe rust and may be responsible for the high susceptibility of cultivars. Cultivars carrying *Yr9* should be restricted to use alone in wheat production and be planted match with other genes (Li *et al*., 2011). In the

early 2000s, this was the case for the stripe rust epidemics in the United States when virulence to *Yr9* occurred in high frequency (Chen, 2005; Chen *et al*., 2002). The two most recent epidemics of stripe rust were due to the successive emergence of *Pst* pathotypes with new virulence factors overcoming the widely used *Yr9* resistance gene (Sharma-Poudyal *et al*., 2013). More than 80% of wheat cultivars released in the late 1980s possessed *Yr9* and, as a result, virulence to *Yr9* occurred in 1985 caused stripe rust epidemics that resulted in yield loss of 2.65 million tons in 1990 in China (Chen *et al*., 2009). Also, the stripe rust epidemic has been reported in 2002 due to the existence of *Yr9* in the wheat cultivars (Wan *et al*., 2004). The basis for losing resistance in varieties carrying *Yr9* has been reported by Hovmoller (2001). As a result of our investigation, it is revealed that *Yr9* was the most identified gene in the majority of the Egyptian wheat cultivars. The wheat cultivars commercially cultivated in Egypt may have affected by the contemporary *Pst* races due to the presence of *Yr9*.

Resistance singularization is the most important factor causing disease epidemics (Zhu et al., 2000). However, hybridization of the commercial wheat cultivars having a high genetic diversity may not be a logical decision in breeding for resistance, because it could hasten the virulence diversity in *Pst* population (Draz et al., 2019). Mono-culturing or use of a single resistant gene should be avoided to escape cultivar susceptibility due to stripe rust. Varieties with different resistant genes should be deployed in different wheat-growing regions to ensure sustainable control against stripe rust. Therefore, genetically diverse varieties with pyramiding resistance genes is an effective strategy for disease control (He et al., 2011). Knowledge of race-specific resistance genes to stripe rust in Egyptian wheat cultivars provides important information in designing control measures to develop the resistant wheat germplasm. The high susceptibility of *Yr9* and its presence in the majority of Egyptian cultivars may have evolved from extensive use of *Yr9*, originating from Petkus rye (Secale cereale), in wheat-breeding programs worldwide (Stubbs, 1985). The emergence and spread of *Yr9*-virulent races caused serious stripe rust outbreaks in many major wheat-growing regions (Wan et al., 2004; Chen, 2005; Chen et al., 2002 & 2009). Our findings suggest that high susceptibility of Egyptian wheat cultivars to stripe rust may be due to the presence of a race-specific gene *Yr9*. Although, cultivars rated moderately resistance in this study may be available options to challenge the disease, however, to escape the infection due to the evolution *Pst* population, new effective genes for resistance should be incorporated in wheat cultivars.

Conflict of interest

The authors declare that they have no competing interests.

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