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Molecular detection of stripe rust resistance genes in some Egyptian bread wheat cultivars and promising spring wheat lines

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Abstract

Stripe rust (yellow rust), caused by *Puccinia striiformis* f.sp. *tritici* (*Pst*), is a serious disease of wheat worldwide. Growing resistant cultivars is the most cost-effective and environmentally friendly approach to control the disease. Fifteen Egyptian wheat cultivars, five advanced lines, and twelve yellow rust (*Yr*) resistance isogenic lines were evaluated against three of the most prevalent *Pst* physiological races 224E132, 224E32, and 151E80 in Egypt. Infection types and diseases severity for all wheat genotypes at seedling and adult plant stages were recorded, during two successive seasons 2017/18 and 2018/19. In addition, three Sequence-Tagged Sites (STS) markers and three Simple Sequence Repeat (SSR) markers were used for detection of (*Yr5*, *Yr15*, and *Yr26*) and (*Yr10*, *Yr17*, and *Yr18*), respectively. The results revealed that Sakha-93, Sakha-95, and Misr-3 were the only wheat cultivars that showed moderated to high resistance against the three rust races, in seedling and adult plant stages in both seasons. All tested breeding lines were showed high to moderated resistance against races mixture at adult stage in both seasons. However, only breeding Line-2 and Line-5 were showed high to moderated resistance against the three races in seedling stage. Also, high resistance in both seedling and adult plant stages were observed on isogenic lines *Yr5* and *Yr15* against tested races. *Yr5* was detected in three wheat genotypes including Sakh-95, Misr-3 and Line-2. *Yr10* was found only in Sakha-93. *Yr17* was existed only in Misr-3 and all breeding lines except Line-1. *Yr15* was detected in seven commercial cultivars but it was not detected in any of the breeding lines. However, *Yr18*, and *Yr26* were found in all tested wheat genotypes. Some of tested lines could be promising source for effective *Yr* resistance genes such as *Yr5*, *Yr17*, *Yr18* and *Yr26*.

Keywords: *Puccinia striiformis* f.sp. *tritici*, *Yr* genes, spring bread wheat cultivars.

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1. Introduction

Wheat is one of the most important cereal crops. It comes directly after corn and rice in terms of world production. It is the most important staple food for 30% of the world population. It occupies more than 220 million hectares of land with 725 million tons production (Anonymous, 2015). More than 65% of world population is mainly relying on wheat as an important source for nutrition. Which provides 21% of the food calories consumed globally, and 20% of the protein to more than 4.5 billion people in 94 developing countries (Braun et al., 2010). In Egypt, wheat is an important staple food with an annual consumption of about 12.4 million tons, of which about 9.4 million tons are grown locally (Rashed et al., 2016). Wheat is liable to be infected by three types of rusts; stem, stripe, and leaf rust. Stripe rust considers the most important diseases in wheat around the world such as Asia, Europe, the Middle East, and Africa (Chen, 2005; McIntosh & Brown 1997). Stripe rust may cause high yield loss, that could reach up to 70% in usual epidemic years and 100% in severe epidemic years (Li & Zeng, 2002). In Egypt, the first epidemic of stripe rust was recognized on wheat varieties Giza 144 at Manzala during 1967/68 (Abdel-Hak et al., 1972). Then starting from 1995 till 1998, it caused a sever loss on wheat cultivars Sakha-69, Giza-163, Gemmeiza-1, and most of the commercial varieties, especially the long-spiked ones, at the Northern governorates in particular. A little bit cases of epiphytotics were recorded during 1999/2000 (Abu El-Naga et al., 2001; 1999; 1997; El-Daoudi et al., 1996). Among all rust management strategies, breeding for resistant cultivars is the most effective, economical, and environment friendly strategy (Line & Chen, 1995). Recently, 70 officially designated and 100 temporarily named stripe rust resistance genes and quantitative trait loci (QTLs) have been reported (Dracatos et al., 2016; Cheng et al., 2014; McIntosh et al., 2013). However, emerging of new races has overcome most of these resistance genes, in different parts of the world, especially when these genes start to be widely used in wheat breeding and production (Chen et al., 2003; Li & Zeng, 2002; He et al., 2001). In Egypt, the source of *Pts* spores is not accurately known. Therefore, pyramiding of various stripe rust resistance genes is required to protect the growing wheat cultivars against the domestic and the new arrival races. On the other hand, the genetic background of most commercial Egyptian wheat cultivars and advanced lines is not well studied, in terms of resistance to stripe rust. The aim of this study is to evaluate the response of 32 wheat genotypes, including Egyptian commercial wheat cultivars and promising breeding lines against three of the most prevalent *Pst* physiological races 224E132, 224E32, and 151E80. Also detecting the presence of six *Yr* resistance genes, *Yr5*, *Yr10*, *Yr15*, *Yr17*, *Yr18*, and *Yr26*in tested wheat genotypes using STS markers and SSR markers.

2. Materials and methods

2.1 Wheat materials

A total of 32 wheat entries (Table 1)

including; 15 contemporary Egyptian wheat cultivars; five of the most promising, advanced breeding lines which were selected upon their performance in the wheat breeding program, at Sakha Agriculture research station, Egypt; and 12 *Yr* isogenic lines were tested for their seedling and adult-plant responses to the most prevalent Egyptian *Pst* races, 224E132, 224E32, and 151E80. In addition to wheat cultivar Avocet S, which is susceptible to most of *Pst* races, was selected as the susceptible control.

Table 1: Pedigrees of Egyptian bread wheat genotypes tested for stripe rust resistance.

Accessions and original sources		Pedigree
Egyptian bread wheat cultivars		
Sakha-08	Cultivar	CIANO-67(SIB)//SONORA-64/KLEIN-RENDIDOR/3/II-8156; INDUS-66/(SIB)NORTENO;
Sakha-93	Cultivar	Sakha 92/TR 810328 S 8871-1S-2S-1S-0S
Sakha-94	Cultivar	Opata/Rayon//Kauz CMBW9043180-OTOPM-3Y-010M-010M-010Y-10M-015Y-0Y
Sakha-95	Cultivar	PASTOR // SITE / MO /3/ CHEN / AEGILOPS SQUARROSA (TAUS) // BCN /4/ WBLL1
Giza-160	Cultivar	(Regent975-11Giza1392)MidaCadetHindi 62
Giza-168	Cultivar	MIL/BUC//Seri CM93046-8M-0Y-0M-2Y-0B
Giza-171	Cultivar	Gemmeiza-9 / Sakha-93
Sids-12	Cultivar	BUC//7C/ALD/5/MAYA74/ON//1160-147/3/BB/GLL/4/CHAT"S"/6/MAYA/VUL-4SD-1SD-1SD-0SD.
Sids-13	Cultivar	KAUZ "S"//TSI/SNB"S". ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP-0SD.
Sids-14	Cultivar	Bow"s"/Vee"s"//Bow's'/Tsi/3/BANI SUEF 1
Gemmeiza-11	Cultivar	BOW ‘‘S’’/KVZ ‘‘S’’//7C/SERI82/3/GIZA168/SKHA61
Gemmeiza-12	Cultivar	BUC//7C/ALD/5/MAYA74/ON//1160-147/3/BB/GLL/4/CHAT ‘‘S’’/6/MAYA/VUL//CMH74A.630//4*SX
Misr-1	Cultivar	OASIS/SKAUZ//4*BCN1312*PASTOR
Misr-2	Cultivar	SKAUZ/BAV92
Misr-3	Cultivar	ATTILA*2/PBW65*2/KACHU
Breeding lines		
Line-1	Breeding Line	FLORKWA -2 /10/ MAYA /YD/6/HK/MDA38/4/4777/3/REI//Y/KT/5/YR/7/KOEL/8/MOR/BOW/9/SERI/11/ATTILA*2/GIZA 168
Line-2	Breeding Line	SIDS1/ ATTILA // GOUMRIA-17 (PBW 343 /6/ SHA7 / VEE#5 /5/ VEE#8 // JUP / BJY /3/ F3.71 / TRM/4 / 2*WEVER)
Line-3	Breeding Line	SW89.5181/KAUZ/4/MILAN/KAUZ//PRINIA/3/BABAX
Line-4	Breeding Line	CHEN/AEGILOPS SQUARROSA(TAUS)/ /BCN/3/2* KAUZ/4/GEN*2 //BUC/ FLK /3/ BUCHIN.
Line-5	Breeding Line	SIDS1/ ATTILA // GOUMRIA-17 (PBW 343 /6/ SHA7 / VEE#5 /5/ VEE#8 // JUP / BJY /3/ F3.71 / TRM/4 / 2*WEVER)

Table 1 (Supplementary): Differential genotypes* used to identify pathotypes of stripe rust incited by *Puccinia striiformis* f.sp. *tritici* in Egypt.

Differential cultivars	Abbreviation	Decanery value	Resistance gene	Type
GI. World differential set				
Chinese 166	Ch	(2^0) =1	*Yr1*	Winter
Lee	Lee	(2^1) = 2	*Yr7*	Spring
HeinesKolben	HK	(2^2) = 4	*Yr2Yr6*	Spring
Vilmorin 23	V23	(2^3) = 8	*Yr3*	Winter
Moro	Mo	(2^4) = 16	*Yr10*	Winter
StrubesDickkopf	Std	(2^5) = 32	*SD*	Winter
Suwon 92 × Omar	Su	(2^6) = 64	*SU*	Winter
Clement	Cl	(2^7) = 128	*Yr2Yr9*	Winter
Tirticumspelta Album	Sp	(2^8) = 256	*Yr5*	Spring
GII. European Differential set				
Hybrid 46	H46	(2^0) =1	*Yr4*	Winter
Reichersberg 42	R42	(2^1) = 2	*Yr(7)*	Winter
Heines Peko	Pe	(2^2) = 4	*Yr2 Yr(6)*	Spring
Nord Desprez	No.D	(2^3) = 8	*Yr(3)*	Winter
Compare	Com	(2^4) = 16	*Yr8*	Spring
Carstens V	CV	(2^5) = 32	*YrCV*	Winter
Spaldings Prolific	Spa	(2^6) = 64	*YrSP*	Winter
Heines VII	HVII	(2^7) = 128	*Yr2*	Winter

* Johnson et al., (1972).

2.2 *Puccinia striiformis* f.sp. *tritici* races

The most prevalent Egyptian *Pst* races 224E132 and 224E32, and the new race 151E80 were identified previously using the World and European group of wheat differential varieties as proposed by Johnson et al. (1972) (Supplementary Table 1). The selected races were carrying different virulence genes that make them a good material for testing the major and adult plant resistance of tested wheat genotypes.

2.3 Seedling evaluation

This experiment was conducted under

greenhouse condition, as described by Li and Zeng (2002). Five seeds of each genotype were planted in plastic pots, with both a diameter and height 10 cm, with five replicates. Seedlings of tested wheat genotypes were inoculated at the two-leaf stage, with the fresh urediniospores of *Pst* races 224E132, 224E32 and 151E80. After inoculation, the seedlings were incubated in a dew chamber at 10-12°C and 100% relative humidity under dark conditions for 12 h. Then, infected seedlings were moved to a separate greenhouse chamber maintained at 12–17°C and seedlings were subjected to 16h light and 8 h darkness. Seedling infection types (IT) were recorded 15–18 days after inoculation, following 0–9 scale (Line & Qayoum, 1992). Plants with IT 0-3 were considered resistant, ITs of 4-6 were considered intermediate, and ITs of 7-9 were considered susceptible.

2.4 Field evaluation

All the tested wheat genotypes were evaluated in adult plant stage, at the experimental farm of Sakha Agriculture research station, Egypt during 2017/2018 and 2018/2019 growing season. The experiment was designed as a randomized complete block. Three replicates were used for each genotype. Each replicate included 3 rows. Approximately 20 seeds, of each wheat genotype, were sown in a 1-m row with 20 cm distance between rows. The susceptible cultivar Morocco was planted in one row for every 20 rows and around the blocks as a stripe rust spreader. During early March, Morocco was inoculated with a mixture of urediniospores of 224E132, 224E32 and 151E80 as described by Li and Zeng (2002). At late tilleringstage all tested wheat genotypes were uniformly dusted with urediniospores of three races' mixture and talcum powder at the rate of 1:25 (v/v).Infection response was scored as described by Roelfs et al. (1992), resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S). Disease severities were assessed based on the percentage of leaf area affected (0, 1, 5, 10, 20, 30, 40, 60, 80 and 100%) (Li & Zeng, 2002). The Coefficient of Infection (CI) for stripe rust were calculated according to Akhtar et al. (2002), by multiplying the response value with the intensity of infection in percent. The response value was determined as following: 0.0 =no disease, 0.2 =R, 0.3 =R to MR, 0.4 =MR, 0.6 =MR-MS, 0.8 =MS, and 0.9 MS-S. Average coefficient of infection (ACI) was derived from the sum of CI values of each entry divided by the number of tested years. The highest ACI of a candidate line is set at 100 and all other lines are adjusted accordingly. This gives the Country Average Relative Percentage Attack (CARPA). The '0' to '9' scale previously designated as Resistance Index (R.I) has been re-designated as RRI (Relative Resistance Index). RRI was calculated from CARPA, on a 0 to 9 scale, where 0 denotes most susceptible and 9 denotes highly resistant (Akhtar et al., 2002). The RRI was calculated according to the following formula:

$$\mathrm{RRI} = \frac{(100 - \mathrm{CARPA})}{100} \times 9$$

2.5 DNA extraction and molecular marker detection of *Yr* genes

All the tested wheat genotypes were

grown in a rust-free greenhouse. Two weeks later approximately 100 mg leaf tissue of each wheat entry was collected. DNA was extracted using a cetyltrimethylammonium bromide (CTAB) method (Saghai-Maroof et al., 1984). The DNA stock solution was adjusted to a concentration of 50 ng/µl with sterilized ddH2O for use as the working solution for the polymerase chain reaction (PCR). The eluted DNA was stored at -20ºC. PCR mixture for *Yr* genes detection. PCR reaction was conducted in reaction volume of 25 µl; contained (1 µl) of 25 ng nucleic acid, 1 µl of each primer (10 pmol), 12.5 µl of Master Mix (Promega Corporation, USA) and 9.5 µl of Nuclease free water (Promega). Three Sequence-Tagged Sites (STS) markers and three Simple Sequence Repeat (SSR) markers were used for detection of (*Yr5*, *Yr15*, and *Yr26*) and (*Yr10*, *Yr17*, and *Yr18*), respectively (Table 2). Then 15 µl of all PCR products were analyzed by electrophoresis through a 1.5% agarose gel, stained with ethidium bromide. DNA bands were visualized using a UV transilluminator. The PCR and electrophoresis of process were carried out as described by Murphy et al. 2009, Singh et al. 2009, Wang et al. 2008, Wen et al. 2008 and Lagudah et al. 2006, 2009 with minor modifications.

Table 2: Sequences of primers, PCR conditions and references for *Yr* marker used to identify six *Yr* genes in wheat cultivars and lines.

Wheat cultivars. Gene	Marker type	Marker name	Primer sequence	GC %	Ta (°C)	Fragment size (bp)	Reference
Yr5	STS	STS7	F5'-GTACAATTCACCTAGAGT-3'	39	45	478	Chen et al. (2003)
		STS8	R5'-GCAAGTTTTCTCCCTAT-3'	41			
Yr10	SSR	Yr10-F11	F5'-TTGGAATTGGCGACAAGCGT-3'	53	64	755	Singh et al. (2009)
		Yr10-R11	R5'-GTGATGATTACCCACTTCCTC-3'	50			
Yr15	STS	Xa1LRF	5´-CTCACTCTCCTGAGAAAATTAC-3´	41	56	700	Murphy et al. (2009)
		PtoFen-S	5´- ATGGGAAGCAAGTATTCAAGGC-3	45			
Yr 17/Sr38/Lr37	SSR	VENTRUIP	F5'-AGGGGCTACTGACCAAGGCT-3'	60	65	259	Helguera et al. (2003)
		LN2	R5'-TGCAGCTACAGCAGTATGTACACAAAA-3'	40			
Yr18/Pm38/Lr34	SSR	L34DINT9-F	F5-'TTGATGAAACCAGTTTTTTTTCTA-3'	25	58	517	Lagudah et al. 2009
		L34PLUS-R	R5'-GCCATTTAACATAATCATGATGGA- 3'	33			
Yr26	STS	WE173	F5'-GGGACAAGGGGAGTTGAAGC-3'	60	55	259	Wang et al. (2008)
			R5'-GAGAGTTCCAAGCAGAACAC-3'	50			

3. Results

3.1 Seedling evaluation

Three *Pst* races 224E132, 224E32, and 151E80 were used for seedling evaluation of 32 wheat genotypes under greenhouse condition (Table 3).Among all tested wheat cultivars only three cultivars Sakha-93, Sakha-95, and Misr-3 were showed high to intermediated resistance against *Pst* races. However, two cultivars Sakh-94 and Gemmiza-12 were showed intimidated resistance to races 224E32 and 151E80, but both cultivars were susceptible to race 224E132. In addition, among five breeding lines only two lines, Line-2 was showed high resistance, while Line-5 was expressed intermediate resistance to the tested *Pst* races. The tested *Yr* NILs varied for their responses to the three races. *Yr5* and *Yr15* were showed high resistance against all tested races, compared with other NILs. However, *Yr1*, *Yr7*, *Yr10*, and *Yr26* were expressed low to intermediate infection types to only two races 224E132 and 224E32, and high infection types for race 151E80. The susceptible cultivar Avocet S was showed high infection types against all races.

3.2 Adult plant stage evaluation

The field experiment was implemented to study the response of wheat genotypes against the mixture of three *Pst* races, (224E132, 224E32, and 151E80), in adult plant stage. According to the obtained data two cultivars Sakha-95 and Misr-3 were showed high resistance to *Pst* races, and low disease severity was observed on both cultivars during 2017/18 and 2018/2019. Also, Sakha-95 and Misr-3 were expressed high RRI value 8.86. Four cultivars Sakha-93, Sakha-94, Misr-1, and Misr-2 were expressed moderated resistance, with low disease severity less than 20, and IRR values between 6.57 and 8.64. However, all other cultivars were showed moderated to high susceptibility to the *Pst* races in both growing seasons 2017/18 and 2018/2019. Four breeding lines, Line-2, Line-3, Line-4, and Line-5, were showed high resistance with disease severity 20 and less, and IRR 8.86 for line-2 and Line-5 and 8.73 for Line-3 and Line-4. However, Line-1 was moderated resistance to the *Pst* races with 30% disease severity, IRR values for both lines were 7.92. Among twelve tested isogenic lines only four lines, *Yr1*, *Yr5*, *Yr10*, and *Yr15*, were expressed high resistance. The same lines were showed low disease severity less than 10, ACI less than 1.5, and IRR ranged from 8.86 to 8.91during 2017/2018 and 2018/2019 growing seasons. Moderated resistance was observed on *Yr18* against *Pst* races with disease severity less than 20 in 2017/2018 and IRR 8.46.

Table 3: Responses of wheat genotypes to three *Puccinia striiformis* f. sp. *tritici* races in seedling and adult plant stages.

Wheat genotype	Seedling stage			Adult stage				CI	ACI	RRI
				2017/18		2018/19				
	224E132	224E32	151E80	IT	DS	IT	DS			
Egyptian wheat cultivars										
Sakha-8	9	9	9	S	70	S	80	150	75	2.25
Sakha-93	5	4	3	MR	5	MS	30	26	13	7.83
Sakha-94	7	6	6	MR	10	MR	10	8	4	8.64
Sakha-95	4	3	4	R	5	R	10	3	1.5	8.86
Giza-160	9	9	9	S	80	S	90	170	85	1.35
Giza-168	6	7	8	MS	20	S	5	21	10.5	8.05
Giza-171	7	8	8	MS	10	S	30	38	19	7.29
Sids-12	9	9	9	S	80	S	80	160	80	1.8
Sids-13	9	9	8	S	70	S	70	140	70	2.7
Sids-14	9	7	7	MS	30	S	40	46	23	6.93
Gemmiza-11	9	9	9	S	70	S	80	150	75	2.25
Gemmiza-12	8	6	6	MS	30	S	30	54	27	6.57
Misr-1	7	8	8	MR	10	S	30	34	17	7.47
Misr-2	7	7	7	MR	20	MS	30	32	16	7.56
Misr-3	3	4	4	R	5	R	10	3	1.5	8.86
Wheat breeding lines										
Line-1	8	8	8	MR	30	MR	30	24	12	7.92
Line-2	3	3	4	R	5	R	10	3	1.5	8.86
Line-3	8	7	8	R	10	R	20	6	3	8.73
Line-4	7	8	8	R	10	R	20	6	3	8.73
Line-5	6	6	6	R	5	R	10	3	1.5	8.86
Yr-NILs										
Yr1	0;	0;	8	R	5	R	5	2	1	8.91
Yr5	0;	1	0;	R	5	R	5	2	1	8.91
Yr6	9	3	8	S	80	S	80	160	80	1.80
Yr7	4	4	9	S	40	S	60	100	50	4.50
Yr9	8	9	8	S	30	S	70	100	50	4.50
Yr10	1	0;	8	R	5	R	10	3	1.5	8.86
Yr15	3	2	3	R	5	R	5	2	1	8.91
Yr17	8	6	7	MS	10	MS	30	32	16	7.56
Yr18	9	8	8	MR	10	MR	20	12	6	8.46
Yr26	3	3	7	MS	20	MS	30	40	20	7.20
Yr27	8	8	9	MS	30	S	20	44	22	7.02
Yr32	7	3	8	S	10	S	30	40	20	7.20
Avocet s	9	9	9	S	90	S	100	190	95	0.45

3.3 Detection of six *Yr* genes in wheat genotypes using molecular markers

Six different markers were used for detecting the presence of six *Yr* genes; *Yr5*, *Yr10*, *Yr15*, *Yr17*, *Yr18* and *Yr26*, in a collection of 15 recent Egyptian wheat cultivars and 5 breeding lines (Table 4) and (Figure 1). The results indicated that *Yr5* was only detected in three wheat genotypes including two cultivars Sakha-95 and Misr-3, one breeding line Line-2. *Yr18*, and *Yr26* were detected in all Egyptian wheat cultivars and breeding lines. However, *Yr10* was detected in only one cultivar Sakha-93. *Yr15* and *Yr17*were detected in some genotypes but not all the tested genotypes.

Table 4: Detection of yellow rust (*Yr*) resistance genes using different molecular markers in Egyptian wheat cultivars and breeding lines.

No.	Wheat accessions	Yr5	Yr10	Yr15	Yr17	Yr18	Yr26
Wheat genotypes							
1	Sakha-8	-	-	-	-	+	+
2	Sakha-93	-	+	+	-	+	+
3	Sakha-94	-	-	-	-	+	+
4	Sakha-95	+	-	-	-	+	+
5	Giza-160	-	-	-	-	+	+
6	Giza-168	-	-	+	-	+	+
7	Giza-171	-	-	+	-	+	+
8	Sids-12	-	-	-	-	+	+
9	Sids-13	-	-	-	-	+	+
10	Sids-14	-	-	+	-	+	+
11	Gemmiza-11	-	-	+	-	+	+
12	Gemmiza-12	-	-	+	-	+	+
13	Misr-1	-	-	+	-	+	+
14	Misr-2	-	-	-	-	+	+
15	Misr-3	+	-	-	+	+	+
Breeding lines							
16	Line-1	-	-	-	-	+	+
17	Line-2	+	-	-	+	+	+
18	Line-3	-	-	-	+	+	+
19	Line-4	-	-	-	+	+	+
20	Line-5	-	-	-	+	+	+

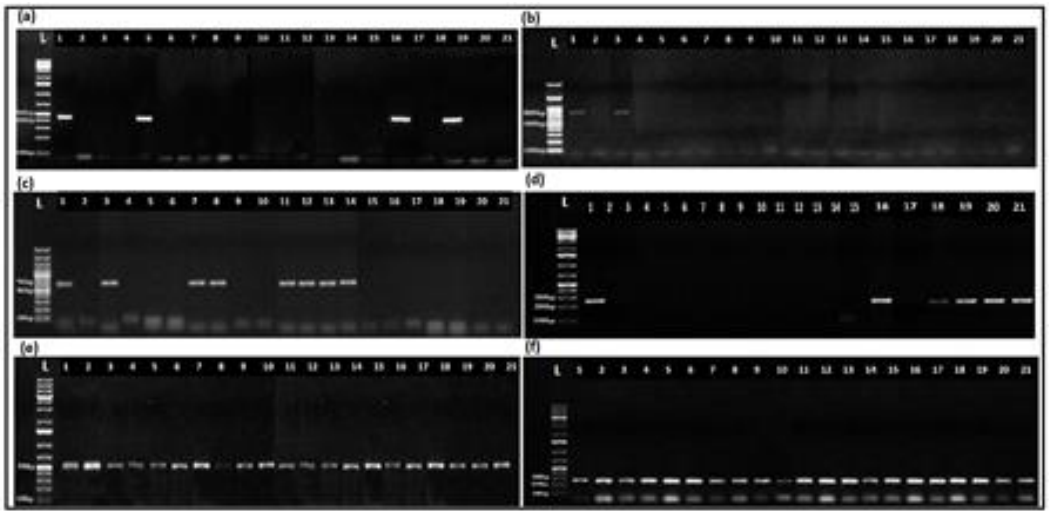

Figure 1: Electrophoretic amplified pattern of DNA extracted from 15 wheat cultivars and five breeding lines using the specific primers of six Yr genes (a) Yr5, (b)Yr10, (c)Yr15, (d)Yr17, (e)Yr18, and (f)Yr26. In all patterns L = DNA Ladder, 1 = positive samples, 2 = Sakha-8, 3 =Sakha-93, 4 = Sakha-94, 5 = Sakha-95, 6 = Giza-160, 7 = Giza-168, 8 = Giza-171, 9 = Sids-12, 10 = Sids=13, 11 = Sids-14, 12 = Gemmiza-11, 13 = Gemmiza-12, 14 = Misr-1, 15 = Misr-2, 16 = Misr-3, 17 = Line-1, 18 = Line-2, 19 = Line-3, 20 = Line-4, 21 = Line-5.

Yr15 was found in seven cultivars, Sakha-93, Giza-168, Giza-171, Sids-14, Gemmiza-11, Gemmiza-12, and Misr-1. However, *Yr15*was not found in any of the tested breeding lines. *Yr17* was detected only in one of the most recent released wheat cultivars, Misr-3. All the tested breeding lines appeared to have *Yr17* except Line-1.

4. Discussion

This study was aimed to evaluate the recent cultivated Egyptian wheat cultivars and some promising wheat breeding lines against the most predominant *Pst* races, 224E132, 224E32, and 151E80.The tested wheat genotypes showed variation in their responses to *Pst* races. Wheat cultivars, Sakha-93, Sakha-95, and Misr-3 were the only wheat cultivars that showed moderated to high resistance against tested *Pst* races in seedling and adult plant stages. The resistance of these cultivars could be due to the presences ofeffective stripe rust genes such as;*Yr5*which found in Sakha-95, and Misr-3, *Yr15* in Sakha-93, and *Yr17* in Misr-3.Stripe rust resistance genes, *Yr5*, *Yr15*, and *Yr17*, are still effective to stripe rust in Egypt (Shahin et al., 2015; Abu-Aly et al., 2014). Only breeding Line-2 was showed high resistance to *Pst* races in seedling and adult plant stages that could be due to the presence of *Yr-5*, 17, 18 and 26 in its genomic background. Also, Line-5 was showed moderated resistance in seedling and high resistance with low disease severity in adult stage, against *Pst* races. In general, all the tested Lines were showed high to moderated resistance against *Pst* races in the field which could be due to presence of adult plant resistance genes. Interestingly, high infection type was observed on *Yr1* which considered one of the most durable *Yr* gene against 151E80 (author observation). However, it expressed high resistance in the open field at adult plant stage. Another study in Iran by Afshari (2018) was reported virulence against *Yr1*, *Yr3*, and *Yrsp*. Therefore, Breeders who relaying on *Yr1* as a major resistance gene at Mediterranean region should be cautious.Sakha-94, Misr-1, Misr-2, and Line-1 showed moderated resistance to the race mixture in adult plant stage. The molecular study indicated that*Yr5*was detected in Sakha-95, Misr-3, and Line-2. While,*Yr18*and *Yr26* were detected in all tested wheat cultivars and lines.*Yr5*was originally identified in hexaploid *Triticum aestivum* ssp. *spelta* cv. *album* (TSA) (Zhang et al., 2009). *Yr5* is an important *Yr* gene. It confers resistance to almost all isolates of *Pst* in the world, except for Australia (Wellings & McIntosh, 1990) and probably India (Nagarajan et al., 1986). *Yr18*/*Lr34*/*Pm38*/*Sr57* is a slow-rusting and mildewing gene that confers partial, durable resistance to multiple fungal pathogens (Wu et al., 2015; Liang et al., 2006; Spielmeyer et al., 2005; McIntosh, 1992; Singh, 1992; Singh & Rajaram, 1992). Therefore, this multi-pathogen resistance locus is a valuable source of resistance in wheat breeding (Urbanovich et al., 2006). The appearance of *Lr34/Yr18/Pm38*, in Egyptian cultivars goes back to the 1970s, when Egyptian breeders began

incorporating the Japanese cultivar Akakomughi (grandparent of spring wheat variety Frontana, used widely as a source of *Lr34*) in the parentage of most released Egyptian cultivars (Basent et al., 2011). The Brazilian cultivar Frontana is the source of *Lr34*/*Yr18* in a significant proportion of CIMMYT Since Sakha-8 is considered the donor of *Lr34* in subsequent crosses which led to many recent varieties such as Sakha-94 and Sids-13(Fahmi et al., 2015). *Yr26* was alleged to be from the *T. turgidum* durum line c80-1 (Ma et al., 2001) and was located on wheat chromosome 1B (Zhang et al., 2013; Wang et al., 2008; Ma et al., 2001). *Yr10* was isolated from Moro, it located in the short arm of chromosome 1B (Singh et al., 2009; Metzger & Silbaugh, 1970). In Egypt, *Yr10* consider an important *Yr* gene, in a study by Abu-aly et al. (2014), it exhibited high level of resistance against 198E56 and 128E28. Also, *Yr10* expressed seedling and adult plant resistance to numerous races tested by Shahin et al. (2015). Among all tested cultivars,*Yr10* was detected in only Sakha-93. This gene needs to be introgressed intocommercial wheat cultivars. The importance of *Yr10* is not solely the response of the gene itself, but also the expression of another genes that combined to *Yr10*such as *Yr8* (Chen et al., 2013). *Yr15* was detected in seven Egyptian wheat cultivars but not in any of the tested breeding lines. It derived from *Triticum dicoccoides*, was located in chromosome 1BS (McIntosh et al., 1996). It is an effective *Yr* gene against most *Pst* races in Egypt (Shahin & Ragab, 2015). For pyramiding a durable resistance to stripe rust in Egypt, *Yr5* and *Yr15*recommendedto be introgressed into the most recent cultivars. *Yr17* is an important *Yr* gene that confers durable resistance. Also, it is linked to *Yr5* and contribute to the resistance pathway of *Yr5* in wheat plants. In addition to *Yr17*,*Lr37*a leaf rust resistance gene, and *Sr38*a stem rust resistance gene is located within a segment of *Triticum ventricosum* chromosome 2NS translocated to the short arm of wheat chromosome 2A (Helguera et al., 2003). Among all tested wheat cultivars only the most recent cultivar Misr-3 has *Yr17*. The low infection type of Misr-3 in seedling stage and the high resistance of adult plants could be due to the presence of *Yr5*, *Yr17*, *Yr18*, and *Yr26*. *Yr17* was found in four spring wheat breeding lines, which could be used as a source of *Yr17*. Despite the detection of *Yr18* in all the tested wheats, most of them expressed high infection types against tested *Pst* races. That could be due to the absences of other *Yr* or functional genes which involve in the final phenotype response *Yr18*. There are a various studies reported that resistances controlled by NBS-LRR genes may not be durable, and resistances controlled by non-NBS-LRR genes are more likely to be durable (Chen et al., 2013; Fu et al., 2009; Krattinger et al., 2009). Where, the final response phenotypes are not only resulted as expression of one gene it could result as a various defense which regulates by numerous *Yr* and functional genes. Therefore, breeders should be cautious about what *Yr* genes could be combined in particular cultivar to express high level of resistance as a final

phenotypic response. In conclusion, six *Yr* genes were detected using different markers. *Yr5*, *Yr18*, *Yr26* were detected in all tested genotypes. The presences of *Yr5*in three wheat genotypes expressed high resistance against all tested *Pst* races in all stages. However, *Yr18* was not successfully achieved high resistance in seedling and adult plant stages in some cultivars due to the absences of important *Yr* genes such as *Yr17* that has a complementary resistance pathway to *Yr18*. There are four promising spring wheat breeding lines could be used as a source for *Yr5*, *Yr17*, *Yr18*, and *Yr26*.

References

Abdel-Hak TM, Stewart DH, Kamel AH, 1972. The current stripe rust situation in the Near East Countries. Regional wheat workshop, Proceedings of The Ford Foundation. Vol. 1-Diseases, Beirut, Lebanon.

Abu El-Naga SA, Khalifa MM, Bassyouni AA, Youssef WA, Shehab El-Dien TM, Abdel Latif AH, 1999. Revised evaluation for Egyptian wheat germplasm against physiologic races of stripe rust (*Puccinia striiformis* West.). Mansoura Journal of Agricultural Science **24**(2): 477–488.

Abu El-Naga SA, Khalifa MM, Sherif S, Youssef WA, El-Daoudi YH, Shafik I, 2001. Virulence of wheat stripe rust races identified in Egypt during 1999/2000 and sources of resistance. First Regional Yellow Rust Conference for Central & West Asia and North Africa, SPH, Karj, Iran.

Abu El-Naga SA, Khalifa MM, Youssef WA, Imbaby IA, El-Shamy MM, Amer E, Shehab El-Dien TM, Shamy MME, 1997. Effect of stripe rust infection on grain yield in certain wheat cultivars and the economic threshold of chemical control application in Egypt during 1996/1997 growing season. National Annual Coordination Meeting, Nile Valley and Red Sea Regional Program, Egypt, pp. 81–90.

Abu-Aly AA, Shahin AA, EL-Naggar DR, Ashmawy MA. 2014. Identification of stripe rust resistance genes *Yr*'s in candidate Egyptian and CIMMYT wheat genotypes by molecular markers. Mansoura Journal of Plant Protection and Pathology **5**(6): 717–727.

Afshari F, 2018. First report of virulence to resistance genes *Yrsp*, *Yr1* and *Yr3* by wheat yellow rust pathogen (*Puccinia striiformis* f.sp. *tritici*). Borlaug Global Rust Initiative (BGRI) Workshop Proceedings, Ithaca, New York, USA. https://www.globalrust.org/content/first-report-virulence-resistance-genes-yrsp-*yr1*-and-*yr3*-wheat-yellow-rust-pathogen-puccinia.

Akhtar MA, Ahmad I, Mirza JI, Rattu AR, E-Ul-Haque, Hakro AA, Jaffery AH, 2002. Evaluation of candidate lines against stripe and leaf rusts under national uniform wheat and barley yield trial 2000-2001. Asian Journal of Plant Sciences **1**: 450–453

Anonymous, 2015. USDA, Economic Research Service, Wheat Data. Online: http://www.ers.usda.gov/data-products/wheat-data.aspx. Accessed 15 September 2015.

Braun HJ, Atlin G, Payne T, 2010. Multi-location testing as a tool to identify plant response to global climate change. Climate Change and Crop Production, C.R.P. Reynolds, ed. CABI, London,

UK, pp. 115–138.

Chen X, Coram T, Huang X, Wang M, Dolezal A. 2013. Understanding molecular mechanisms of durable and non-durable resistance to stripe rust in wheat using a transcriptomics approach. Current Genomics **14**(2): 111–126.

Chen XM, 2005. Epidemiology and control of stripe rust (*Puccinia striiformis* f.sp. *tritici*) on wheat. Canadian Journal of Plant Pathology **27**: 314–337.

Chen XM, Ling P, Wood DA, Moore MK, Pahalawatta V, 2003. Epidemiology and control of wheat stripe rust in the United State. Annual Wheat Newsletter **50**: 274–276.

Cheng P, Xu LS, Wang MN, See DR, Chen XM, 2014. Molecular mapping of genes *Yr64* and *Yr65* for stripe rust resistance in hexaploid derivatives of durum wheat accessions PI 331260 and PI 480016. Theoretical and Applied Genetics **127**: 2267–2277.

Dracatos PM, Zhang P, Park RF, McIntosh RA, Wellings CR, 2016. Complementary resistance genes in wheat selection 'Avocet R' confer resistance to stripe rust. Theoretical and Applied Genetics **129**: 65–76.

El-Daoudi YH, Ikhlas S, Enayat HG, Abu El-Naga S, Mitkees R, Sherif S,Khalifa M, Bassiouni AA, 1996. Stripe rust occurrence in Egypt and assessment of grain yield losses in 1995. Proceedings d Symposium Regional surles Maladies des Cereales et des Legumineuses Alimentaires, pp. 341–351.

Fahmi AI, El-Shehawi AM, El-Orabey WM, 2015. Leaf rust resistance and molecular identification of *Lr34* gene in Egyptian wheat. Microbial & Biochemical Technology **7**(6): 338–343

Fu DL, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen XM, Sela H, Fahima T, Dubcovsky J. 2009. A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science **323**: 1357–1360.

He ZH, Rajaram S, Xin ZY, Zhang GZ, 2001. A history of wheat breeding in China. International Maize and Wheat Improvement Center, El Batán, Mexico, Mexico.

Helguera M, Khan IA, Kolmer J, Lijavetzky D, Zhong-qi J, Dubcovsky L. 2003. PCR assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop Science **43**: 1839–1847.

Johnson R, Stubbs RW, Fuch E, Chamberlain NH, 1972. Nomenclature for physiologic races of *P. striifoormis* infecting wheat. Transactions of the British Mycological Society **58**: 475–480.

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller BA, 2009. putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science **323**: 1360–1363.

Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J, Spielmeyer W, Brown-Guedira G, Selter LL, Keller B, 2009. Gene-specific markers for the wheat gene *Lr34*/*Yr18*/*Pm38* which confers resistance to multiple fungal pathogens. Theoretical and Applied Genetics **119**: 889–898.

Lagudah ES, McFadden H, Singh RP,

Huerta-Espino J, Bariana HS, Spielmeyer W. 2006. Molecular genetic characterization of the *Lr34*/*Yr18* slow rusting resistance gene region in wheat. Theoretical and Applied Genetics **114**: 21–30.

Li ZQ, Zeng SM, 2002. Wheat Rust in China. Chinese Agricultural Press, Beijing, China.

Liang SS, Suenaga K, He ZH, Wang ZL, Liu HY, Wang DS, Singh RP, Sourdille P, Xia XC, 2006. Quantitative trait loci mapping for adult-plant resistance to powdery mildew in bread wheat. Phytopathology **96**: 784–789.

Line RF, Chen XM. 1995. Successes in breeding for and managing durable resistance to wheat rusts. Plant Disease **79**: 1254–1255.

Line RF, Qayoum A, 1992. Virulence, aggressiveness, evolution, and distribution of races of *Puccinia striiformis* (The cause of stripe rust of wheat) in North America. Technical Bulletin No. 1788, US Department of Agriculture, Washington DC, USA, pp. 1967–87.

Ma J, Zhou R, Dong Y, Wang L, Wang X, Jia J, 2001. Molecular mapping and detection of the yellow rust resistance gene *Yr26* in wheat transferred from *Triticum turgidum* L. using microsatellite markers. Euphytica **120**: 219–226

McIntosh RA, 1992. Close genetic linkage of genes conferring adult-plant resistance to leaf rust and stripe rust in wheat. Plant Pathology **41**: 523–527.

McIntosh RA, Brown GN, 1997. Anticipatory breeding for resistance to rust diseases in wheat. Annual Review of Phytopathology **35**: 311–326.

McIntosh RA, Silk J, The TT, 1996. Cytogenetic studies in wheat XVII monosomic analysis and linkage relationships of gene *Yr15* for resistance to stripe rust. Euphytica **89**: 395–399.

McIntosh RA, Yamazaki Y, Dubcovsky J, Rogers J, Morris C, Appels R, Xia XC, 2013. Catalogue of gene symbols for wheat. In: 2th International Wheat Genetic Symposium, Yokohama, Japan. Internet Resource: http://www.higen.nig.ac.jp/wheat/komugi/genes/download.jsp (verified Jan 8, 2014).

Metzger RJ, Silbaugh BA, 1970. Inheritance of resistance to stripe rust and its association with glume colour in *Triticum aestivum* L ‘PI 178383’. Crop Science **10**: 567–568.

Nagarajan S, Nayar SK, Bahadur P, 1986. Race 13 (67 S8) virulent on *Triticum spelta*var. *album* in India. Plant Disease **70**: 173.

Rashed MA, Atta AH, Shehab El-Din TM, Mostafa AM, 2016. Development of SSR & STS molecular markers associated with stem rust resistance in bread wheat (*Triticum aestivum*l.). Egyptian Journal of Genetics And Cytology **45**: 261–278.

Roelfs AP, Singh RP, Saari EE, 1992. Stripe rust. In: Rust diseases of wheat: Concept and methods of disease management. G. P. Hettel (Ed.). International Maize and Wheat Improvement Center, Mexico, pp. 23–24.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW, 1984. Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National

Academy of Sciences of the United States of America **81**: 8014–8019.

Shahin AA, RagabKE. 2015. Inheritance of adult plant stripe rust resistance in wheat cultivars Giza160 and Giza168. Mansoura Journal of Plant Protection and Pathology **6**(4): 587–596.

Shahin AA, Shaheen S, Abu Aly AA, 2015. Virulence and diversity of wheat stripe rust pathogen in Egypt. Journal of American Science **11**(6): 47–52

Singh R, Datta D, Priyamvada, Singh S, Tiwari R, 2009. A diagnostic PCR based assay for stripe rust resistance gene *Yr10* in wheat. Acta Phytopathologica et Entomologica Hungarica **44**(1): 11–18.

Singh RP, 1992. Genetic association of leaf rust resistance gene *Lr34* with adult plant resistance to stripe rust in bread wheat. Phytopathology **82**: 835–838

Singh RP, Rajaram S,1992. Genetics of adult-plant resistance of leaf rust in 'frontana' and three CIMMYT wheats. Genome **35**(1): 24–31.

Spielmeyer W, McIntosh RA, Kolmer J, Lagudah ES, 2005. Powdery mildew resistance and *Lr34*/*Yr18* genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat. Theoretical and Applied Genetics **111**: 731–735.

Urbanovich OY, Malyshev SV, Dolmatovich TV, Kartel NA, 2006. Identification of leaf rust resistance genes in wheat (*Triticum aestivum* L.) cultivars using molecular markers. Russian Journal of Genetics **42**: 675–683.

Wang CM, Zhang YP, Han DJ, Kang ZS, Li GP, Cao AZ, Chen PD, 2008. SSR and STS markers for wheat stripe rust resistance gene *Yr26*. Euphytica **159**: 359–366.

Wellings CR, McIntosh RA, 1990. *Puccinia striiformis*f. sp. *tritici* in Australia: pathogenic changes during the wrest 10 years. Plant Pathology **39**: 316–325.

Wen WE, Li GQ, He ZH, Yang WY, Xu ML, Xia XC, 2008. Development of an STS marker tightly linked to *Yr26*against wheat stripe rust using the resistance gene-analog polymorphism (RGAP) technique. Molecular Breeding **22**: 507–515.

Wu L, Xia X, Rosewarne GM, Zhu H, Li S, Zhang Z, He Z, 2015. Stripe rust resistance gene *Yr18* and its suppressor gene in Chinese wheat landraces. Plant Breeding **134**: 634–640.

Zhang P, McIntosh RA, Hoxha S, Dong C, 2009. Wheat stripe rust resistance genes *Yr5* and *Yr7* are allelic. Theoretical and Applied Genetics **120**(1): 25–29.

Zhang X, Han D, Zeng Q, Duan Y, Yuan F, Shi J, Wang Q, WuJ, Huang L, Kang Z, 2013. Fine mapping of wheat stripe rust resistance gene *Yr26* based on collinearity of wheat with *Brachypodium distachyon* and rice. PLoS One **8**: e57885.