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IDENTIFICATION OF STRIPE RUST RESISTANCE GENES *Yr's* IN CANDIDATE EGYPTIAN AND CIMMYT WHEAT GENOTYPES BY MOLECULAR MARKERS

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ABSTRACT

Stripe rust is a widespread damaging disease of wheat, causing significant losses in yield and quality. Each monogenic line of *Yr1, Yr5, Yr10, Yr15, Yr17, Yr32* and *YrSP* exhibited high levels of resistance to both 198E56 and 128E28 races, at seedling stage. The same monogenic lines showed adult plant resistance. Whereas, those with *Yr17* and *YrSP* showed a disease severity ranged between 5MR to 10MR. The monogenic line (*YrSK*) was the only one which recorded susceptible and highly resistant reactions, at seedling and adult stages respectively. Nine Egyptian cultivars and four lines from CIMMYT tested at seedling stage were susceptible (IT 6-8). Misr-1, Misr-2, Gemmezia-10, Line-6043, Line-6085, Line-6086 and Line-6107 showed a range of adult plant responses of TrR–10MR. The remainder had variable degrees of susceptibility (5MS-30S). Fourteen genotypes were screened with three DNA markers to detect the presence of *Yr9*, *Yr17* and *Yr18*. The 1100bp band diagnostic for *Yr9* was present in 7 genotypes, *i.e.* Misr-1, Misr-2, Sids-12, Sids-13, Gemmeiza-9, Gemmeiza-10 and Gemmeiza-11. At 252bp fragment in Line 6043 (8STEMRRS) from CIMMYT was only indicative of *Yr17*, and *Yr18* was present all tested Egyptian and CIMMYT genotypes at 517bp.
Keywords: wheat, stripe rust, resistance gene, molecular marker.

INTRODACTION

Stripe rust of wheat, caused by *Puccinia striiformis f. sp. tritici*, is one of the most widespread and damaging diseases of wheat, causing great losses in yield and grain quality (Line, 2002; Chen, 2005). Grain losses caused by this devastating pathogen have been reported to be 10-70 percent (Chen, 2005).

The frequency of epidemics and damage caused by stripe rust is different in each country. In Egypt stripe rust is the most common and important wheat disease. It caused severe losses in grain yield (Abu El-Naga, *et al*., 2001). Lot of methods are available to control wheat rusts. One of them, the economical and environmentally safe protection of wheat against rusts, is possible by growing resistant wheat varieties. Otherwise, the most feasible method is host genetic resistance to control stripe rust. Utilization of genetic resistance is economical and carries no health and environmental hazards (Chen and Zhao, 2007).

To date more than forty three resistant genes at different loci have been designated and mapped to different wheat chromosomes. Most of genes are race- specific and cultivars possessing some of them played

important role in wheat breeding (Cao *et al*., 2001; McIntosh *et al*., 1996, 2004 and 2005). So far, studies have shown that *Yr11, Yr12, Yr13, Yr14, Yr16, Yr18, Yr29, Yr30, Yr34, Yr36, Yr39, Yr46, Yr49* and *Yr52* are adult-plant resistance genes (Chen, 2005; Bariana *et al*., 2006; Cheng, 2008; Lowe *et al.,* 2011; Ren *et al*., 2012), of which *Yr18* and *Yr36* have been cloned (Fu *et al*., 2009; Krattinger *et al*., 2009). Because adult-plant resistance (APR) genes only have partial effects when present alone, the resistance conferred by them may be incomplete.

The development of molecular markers for specific stripe genes allows the rust detection of these genes independently of the phenotype. Molecular markers can be used in marker-assisted selection for an efficient combination of genes in the pyramiding strategy to create a more durable resistance (Feuillet *et al*., 1995). Simple sequence repeats (SSR) or micro satellites are useful tools for molecular genetic analysis as they are more abundant and display higher levels of polymorphisms in many plant species (Hitta *et al*., 1995 and Plaschke *et al*., 1995). SSR markers *i.e. Yr5, Yr10, Yr15, Yr24* and *YrH52* have been reported for several stripe rust resistance genes (Peng *et al*., 1999, 2000; Sun *et al*., 2002; Wang *et al*., 2002; Zakari *et al*., 2003). Some markers have been used in marker-assisted selection and for pyramiding resistance genes as well as for understanding of the relationships among different genes.

Molecular markers not only allowed the easy and reliable identification of clones and breeding lines but also facilitated the monitoring of introgression and the estimation of genetic diversity and relatedness among germ plasma (Mukhtar *et al*., 2002). To date, many genes have been identified and located by SSR such as *Yr5* (Sun *et al*., 2002), *Yr17* (Robert *et al.*, 2000), *YrH52* (Peng *et al*., 1999 & 2000-a and b), *Yr26* (Ma *et al*., 2001), *Yr15* (Sun *et al*., 1997), *Yr28* (Singh *et al*., 2000) and *Yr32* (Eriksen *et al*., 2000).

This study mainly aimed to identify the stripe rust resistance genes in some wheat cultivars from Egypt and promising lines from CIMMYT by traditional work and advanced method, molecular markers.

MATERIALS AND METHODS

1. Plant material:

In this study, different wheat genotypes were used containing fourteen stripe rust resistance genes (*Yr1, Yr5, Yr6, Yr7, Yr8, Yr9, Yr10, Yr15, Yr17, Yr18, Yr27, Yr32, YrSP,* and *YrSK*), nine Egyptian wheat cultivars (Misr-1, Misr-2, Gemmiza-9, Gemmiza-10, Gemmiza-11, Sakha-93, Sids-12, Sids-13 and Giza-171) and four promising wheat lines (6043, 6085, 6086 and 6107) from international trails nurseries obtained from the International Maize and Wheat Improvement Center (CIMMYT).

2. Evaluation for stripe rust resistance:

Wheat genotypes were tested for stripe rust resistance at seedling and adult plant stages.

Seedling stage:

All plant materials were grown in plastic pots (10 cm in diameter) in greenhouse in Sakha Agriculture Research Station. Each pot contained four entries clockwise in each corner. The method of inoculation was carried out as described by (Stakman *et al*., 1962). The inoculated plants were incubated in a dark dew chamber overnight at 10ºC and 95% relative humidity then moved to the benches in the greenhouse and maintained at 12ºC-15ºC and 95-100% relative humidity. Light intensity was adjusted at 7600 lux in a photoperiod of 16 hours light and 8 hours dark (Stubbs, 1988). After approximately two weeks from inoculation, Infection types (IT's) on the plants (0 - 9) were scored as described by (McNeal *et al*., 1971). Plants with IT's of 0, 0;, 1, 2, 3, 4 and 5 were considered as resistant response, while IT's of 6, 7, 8 and 9 were considered as susceptible response.

Adult stage:

Adult plant resistance was evaluated on the same set of materials in field during 2012/2013 and 2013/2013 growing seasons at Sakha agricultural research station, the recommended agricultural practices were applied. The inoculation of plants was carried out at booting stage according to the method of Tervet & Cassell (1951). Disease severity was assessed using the modified Cobb's Scale (Peterson *et al.,* 1948). Infection response was scored as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S), as described by Roelfs *et al.*, (1992).

Molecular markers:

DNA extraction:

Total genomic DNA of each wheat cultivar and lines was extracted from leaves following the protocol described by Mago *et al*., (2002). Samples of 60 mg leaf tissue were digested in liquid nitrogen with a mortar and pestle using i-genomic plant DNA extraction Mini Kit (iNtRON Biotechnology, Inc, Cat. No. 17371) according to manufacturer's instructions. The eluted DNA was stored at -20 ºC.

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 GoTag®Colorless Master Mix (Promega Corporation, USA) and 9.5 µl of Nuclease free water (Promega). 15 µl of all PCR products were analyzed by electrophoresis through a 1.5% agarose gel, stained with ethidium bromide, and DNA bands were visualized using a UV transilluminator. Sequences of primers are listed in Table (1).

Table (1): List of the tested stripe rust resistance genes and their sequences of primers

***Yr* gene**	**Sequence or Primer Pair**	**Reference**
Yr9	F5' CTCTGTGGATAGTTACTTGATCGA 3' R5' CCTAGAACATGCATGGCTGTTACA 3'	Mago *et al*., 2002
Yr17	F5'AGG GGC TAC TGA CCA AGG CT 3' R5'TGCAGCTACAGCAGTATGTACACAAAA 3'	Helguera *et al*., 2003
Yr18	F5'TTGATGAAACCAGTTTTTTTTCTA3' R5' GCCATTTAACATAATCATGATGGA 3'	Lagudah *et al.,* 2009

RESULTS

Data in Table (2) showed that *Yr1, Yr5, Yr10, Yr15, Yr17, Yr32* and *YrSP*, each at seedling and adult stages, exhibited high resistance against stripe rust reaction. At seedling stage, IT's for 198E56 and 128E28 races were ranged between 0-2. At adult stage, *Yr1, Yr5, Yr10, Yr15* and *Yr32* recorded complete resistance. While *Yr17* and *YrSP* showed a disease severity ranged between 5MR to 10MR.

Also, Table (2) indicated that single gene lines *i.e. Yr6, Yr7, Yr8, Yr9* and *Yr18* were susceptible (IT = 6-9) at seedling stage and were from 10MS to 70S, at adult stage. The monogenic line (*YrSK*) was the only one which recorded susceptible and highly resistant reactions, at seedling and adult stages respectively. Misr-1, Misr-2, Gemmezia-10, Line-6043, Line-6085, Line-6086 and Line-6107 showed a range of adult plant responses of TrR–10MR. The remainder had variable degrees of susceptibility (5MS-30S).

Table (2): Seedling response of monogenic lines, some Egyptian and CIMMYT wheat genotypes against two *Puccinia striiforms* races and their adult plant field reaction during 2012/2013 and 2013/2014 growing seasons.

Genotype	Seedling infection type		Disease severity at the adult stage	
	Race 128E28	Race 198E56	Second season	First season
*Yr1/6*Avocet S*	0	0	0	0
*Yr5/6*Avocet S*	0	0	0	0
*Yr6/6*Avocet S*	7	8	50S	60S
*Yr7/6*Avocet S*	7	9	70S	70S
*Yr8/6*Avocet S*	7	8	40S	60S
*Yr9/6*Avocet S*	6	7	40S	40S
*Yr10/6*Avocet S*	0	2	0	0
*Yr15/6*Avocet S*	0	0	0	0
*Yr17/6*Avocet S*	1	2	5MR	10MR
*Yr18/6*Avocet S*	6	7	10MS	10S
*Yr27/6*Avocet S*	3	4	5 MS	5 MS
*Yr32/6*Avocet S*	2	2	0	0
*YrSP/6*Avocet S*	2	2	10MR	10MR
*YrSK/6*Avocet S*	7	8	5MR	10MR
Misr 1	7	8	5 MR	5 MR
Misr 2	7	7	5 MR	10 MR
Gemmeiza 9	8	7	10S	5MS
Gemmeiza 10	7	8	10 MR	10 MR
Gemmeiza 11	7	7	10 MS	10 MS
Sakha 93	8	7	20MS	30S
Sids 12	7	7	10MS	30S
Sids 13	7	7	10MS	10MS
Giza 171	6	6	5MR-MS	5MS
Line 6043	6	6	TrR	5R
Line 6085	7	7	10MR	5MR
Line 6086	8	7	10R	5R
Line 6107	6	7	5MR	TrMR

Thirteen genotypes were screened with three DNA markers aimed at detecting the presence of *Yr9*, *Yr17* and *Yr18*. Figure (1) showed the polymorphic survey of *Yr9* gene marker which was identified as a fragment of 1100bp band in 7 genotypes (Misr-1, Misr-2, Sids-12, Sids-13, Gemmeiza-9, Gemmeiza-10 and Gemmeiza-11). The only indicative band for *Yr17* was observed at 252 pb fragment Line-6043 (8STEMRRS), as shown in Fig. (2).

While, *Yr18* was identified as a fragment of 517 pb in all tested Egyptian and CIMMYT genotypes, Fig. (3). Data obtained from figures 1, 2 and 3 were summarized in Table (3).

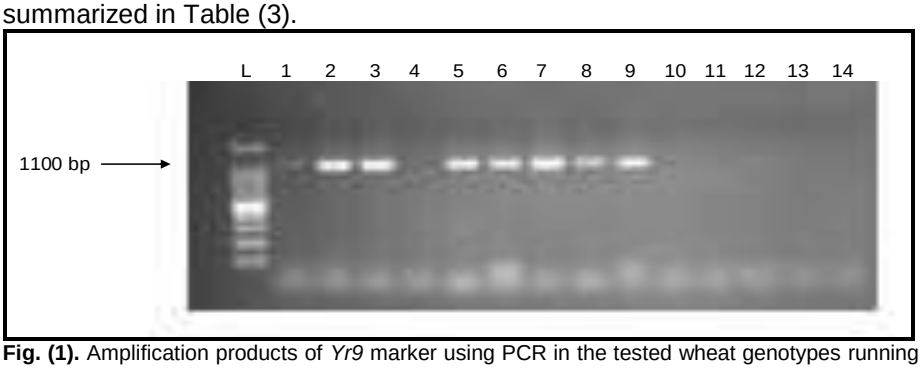

Fig. (1). Amplification products of *Yr9* marker using PCR in the tested wheat genotypes running on agarose gel. L: DNA Ladder, Lane (1): monogenic *Yr9*, (2): Misr-1, (3): Misr-2, (4): Sakha-93, (5): Sids-12, (6): Sids-13, (7): Gemmiza-9, (8): Gemmiza-10, (9): Gemmiza-11, (10): Giza-171, (11): Line-6043, (12): Line-6085, (13): Line-6086 and (14): Line-6107. The arrow shows the fragment which is associated with *Yr9* at 1100bp.

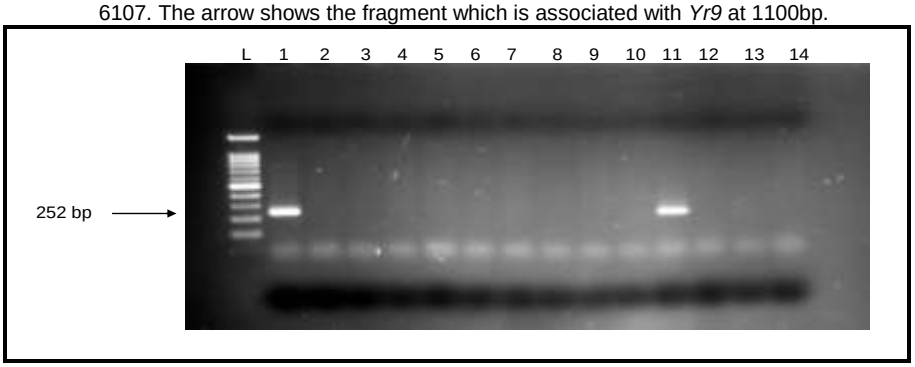

Fig. (2). Amplification products of *Yr17* marker using PCR in the tested wheat genotypes running on agarose gel. L: DNA Ladder, Lane (1): monogenic *Yr9*, (2): Misr-1, (3): Misr-2, (4): Sakha-93, (5): Sids-12, (6): Sids-13, (7): Gemmiza-9, (8): Gemmiza-10, (9): Gemmiza-11, (10): Giza-171, (11): Line-6043, (12): Line-6085, (13): Line-6086 and (14): Line-6107. The arrow shows the fragment which is associated with *Yr17* at 252bp.

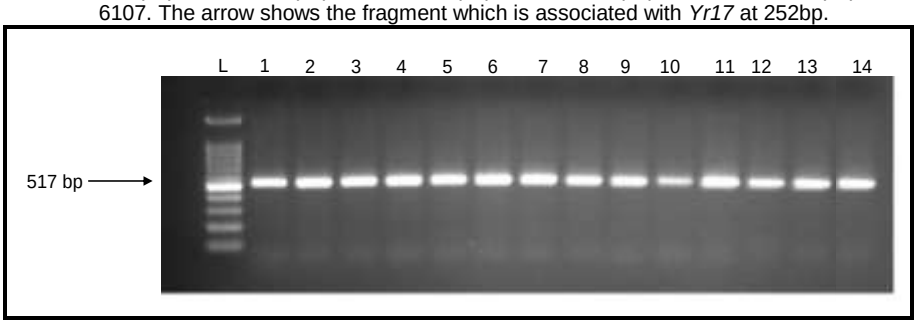

Fig. (3). Amplification products of *Yr18* marker using PCR in the tested wheat genotypes running on agarose gel. L: DNA Ladder, Lane (1): monogenic *Yr9*, (2): Misr-1, (3): Misr-2, (4): Sakha-93, (5): Sids-12, (6): Sids-13, (7): Gemmiza-9, (8): Gemmiza-10, (9): Gemmiza-11, (10): Giza-171, (11): Line-6043, (12): Line-6085, (13): Line-6086 and (14): Line-6107. The arrow shows the fragment which is associated with *Yr18* at 517bp.

Table (3): *Yr* genes detected with PCR based markers in nine Egyptian wheat cultivars and four CIMMYT wheat lines.

No.	Genotypes	*Yr9*	*Yr17*	*Yr18*
1	Misr-1	+	-	+
2	Misr-2	+	-	+
3	Gemmeiza-9	+	-	+
4	Gemmeiza-10	+	-	+
5	Gemmeiza-11	+	-	+
6	Sakha-93	-	-	+
7	Sids-12	+	-	+
8	Sids-13	+		+
9	Giza-171	-	-	+
10	Line-6043	-	+	+
11	Line-6086	-	-	+
12	Line-6107	-	-	+
13	Line-6085	-	-	+

DISCUSSION

Stripe rust is one of the most important diseases of wheat occurring almost in all wheat-producing regions causing crop yield reduction. In order to produce resistant cultivars, it is necessary to identify resistance genes in different germplasms. The objective of this study was to develop a set of public PCR assays for an efficient selection of resistance genes in wheat breeding programs.

The *Yr9* gene was identified in the tested cultivars but was not effective in Egypt. The 1100 bp band diagnostic for *Yr9* was present in 7 genotypes *i.e.* Misr-1, Misr-2, Sids-12, Sids-13, Gemmeiza-9, Gemmeiza-10 and Gemmeiza-11. Due to the lack of pairing between the wheat and rye chromatin (1B and 1BL.1RS) in the wheat background, Sec-1 acts as a marker for Lr26, *Yr9* and Sr31. Studies done by Afshari (2004) indicated that the eight wheat cultivars, except for Alamoot cultivar, have shown good levels of stripe rust resistance in Iran. The 1BL.1RS segment carries genes for resistance to three rusts, namely *Lr26*, *Yr9*, *Sr26*, and gene *Pm8* for resistance to powdery mildew (Zeller 1973).

To date, a number of resistance genes have been introduced into wheat from relatives (Friebe *et al*., 1996; Fedak, 1999). Wheat stripe rust resistance gene, *Yr17*, in combining with Lr37 (for resistance to leaf rust caused by *Puccinia triticina* Eriks) and Sr38 (for resistance stem rust caused by *Puccinia graminis f. sp*. *tritici* Eriks. & E. Henn.) were initially introgressed in wheat variety 'VPM1' from Aegilops ventricosa Ces. (Maia, 1967) and were located on a 2NS/2AS translocation (Bariana and McIntosh, 1993 & 1994). Although new emerged rust races with virulence to *Yr17* have been identified in different countries, this gene in combination with other rust resistance. At 252bp fragment in Line 6043 (8STEMRRS) from CIMMYT was only indicative of *Yr17*.

The adult plant resistance gene to stripe rust (*Yr18*) has also located on the same chromosome segment containing the *Lr34* gene and is tightly linked with it (McIntosh, 1992; Singh, 1992). Additionally, their co-segregation

with other traits such as leaf tip necrosis (Ltn1), powdery mildew resistance gene (Pm38), and tolerance to barley yellow dwarf virus (Bdv1) has been reported (McIntosh 1992; Liang *et al., 2006*; Singh, 1992; Spielmeyer *et al*., 2005). Therefore, this multi-pathogen resistance locus is a valuable source of resistance in wheat breeding (Urbanovich *et al*., 2006). The use of the slow rusting gene pair *Lr34/Yr18* in combination with other slow rusting genes has been suggested to contribute to near immunity to leaf and stripe rust infections (Singh *et al*., 2000). In our finding *Yr18* was present in all tested Egyptian and CIMMYT genotypes.

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تعريف جينات المقاومة للصدأ الأصفر في بعض التراكيب الوراثية المصرية والسيمت المختارة بواسطة الدلائل الجزئية

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يعتبر مرض الصدأ الأصفر على نبات القمح من الأمراض واسعة الانتشار والذي يسبب خسائر كبيرة فى المحصول وجودته . أظهرت النتائج ان المدخلات الوراثية احادية الجين *Yr1، Yr5، Yr10، Yr15، Yr17، Yr32 وYrSP* تظهر مستويات عالية من المقاومة ضد السلالتين 198E56 و 128E8 من الفطر بكسينيا سترايفورمس في طور البادرة وطور البلوغ أيضا تلاحظ وجود مقاومة فى تلك المدخلات . أظهرت المدخلات الوراثية احادية الجين*Yr17* و *YrSP* مقاومة تراوحت من 5MR-10MR ، كما لوحظ أن المدخل الوراثي *YrSK* كان المدخل الوحيد الذي أعطي صفة القابلية للإصابة في طور البادرة وصفة المقاومة العالية في طور البلوغ. على الجانب الأخر وجد ان باقي المدخلات الوراثية المختبرة أظهرت قابلتها للإصابة في طور البادرة تراوحت الشدة المرضية بين 7-9 فى طور البادرة وفى طور البلوغ تراوحت بين 5MS-70S.

كما تم اختبار تسعة أصناف تجارية مصرية وأربعة مدخلات وراثية من السيميت أظهرت قابليتها للإصابة في طور البادرة حيث تراوحت شدة المرض ما بين 6 إلى 8. بينما أظهر كلا من الصنف التجاري مصر-1 ومصر-2 و جميزة-10 والمدخلات الوراثية 6043 و6085 و6086 و6107 مقاومة في طور النبات البالغ تراوحت بين TRR و 10MR ، وأظهرت باقي الأصناف المختبرة درجات متفاوتة من القابلية للإصابة تراوحت بين 5MS و 30S.

كما تم فحص ثلاثة عشر مدخلا وراثياً مع ثلاثة دلائل جزئية بهدف الكشف عن وجود جينات المقاومة للصدأ الأصفر وهى *Yr9* و *Yr17* و *Yr18* حيث وجد أنه عند 1100 (band) وهو النطاق التشخيصي لجين *Yr9* كان موجود فى سبعة من التراكيب الوراثية هي مصر 1و مصر 2 و سدس 12 و سدس 13 وجميزة 9 وجميزة 10 و جميزة 11 .بينما عند bp252 اظهر مدخل وراثي واحد من السيميت هو المدخل رقم 6043 (8STEMRRSN) عن وجود *Yr17* وغيابه فى باقى المدخلات . بينما *Yr18* أظهرت الدلائل الجزئية عن وجودة في جميع التراكيب الوراثية المختبرة من مصر والسيميت . وتعتبر هذه النتائج مهمة لدراسة المقاومة خاصة في المدخلات الغير معروف بها جينات المقاومة والتي تساعد المربي في الحصول على أصناف مقاومة لإدخالها في برامج التربية.

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