

IDENTIFICATION AND EVALUATION OF RESISTANCE GENES SOURCES OF STEM RUST IN DIFFERENT EGYPTIAN AND CIMMYT WHEAT GENOTYPES USING CONVENTIONAL AND MOLECULAR TECHNIQUES.

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ABSTRACT

Wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is one of the most destructive diseases of wheat. Resistant varieties can be the simplest, practical, effective and economical method of plant disease control. Development of molecular markers helps to determine stem rust resistance genes (*Sr* genes). The objective of this study was to identify resistance effective genes against the stem rust at seedling and adult stages and to identify *Sr* genes in eleven genotypes of wheat by molecular markers response of monogenic lines and genotypes of wheat to stem rust reaction. Data at seedling and field studies clustered the genotypes into 4 main categories; (1) resistant at both seedling and adult plant stages monogenic lines, *i.e.* (*Sr24*, *Sr25*, *Sr26*, *Sr31* and *Sr32*), (2) resistant only at adult stage monogenic lines *i.e.* (*Sr27*), (3) scored high susceptibility at both adult and seedling stages monogenic lines (*Sr6*, *Sr16*, *Sr17*, *Sr18*, *Sr21*, *Sr22*, *Sr23*, *Sr28*, *Sr29*, *Sr30*, *Sr33*, *Sr34*, *Sr35*, *Sr36* and *Sr40* and (4) resistant only at seedling stage monogenic line *i.e.* (*Sr37*, *Sr38* and *Sr39*). On the other hand, Egyptian cultivars of Sids-13, Gemmeiza-11 and 3 of CIMMYT-8STEMRRSN Lines *i.e.* line-6043, line -6085 and line-6086 showed resistance at both stages. Meanwhile, Sids-12, Gemmeiza-10, Misr-1 and Misr-2 were resistant at seedling but susceptible at adult plant stage. The rest of the cultivars were susceptible at both stages. In this study, eleven genotypes of wheat were screened with four DNA markers to detect the presence of stem rust resistance genes *Sr2*, *Sr24*, *Sr26* and *Sr31*. Stem rust resistance genes *Sr2* were present in all varieties tested whereas, *Sr24* detected in two local Egyptian cultivar (Sakha 93 and Misr-1) and one line-6085 from (CIMMYT). *Sr26* gene markers produced a 250-bp band that observed in 9 genotypes and did not shown in 2 genotypes. *Sr31*, marker resulted in a 1100-bp fragment in 7 genotypes, this fragment was absent in the remaining 4 genotypes. These results form a basis for mode of resistance study especially to the unknown sources and to mapping those using molecular markers.

Keywords: Stem rust, molecular marker, identifies, resistance genes, susceptibility.

INTRODUCTION

Under favorable conditions, stem rust epidemic have resulted in as much as 50% yield losses, whereas yield losses due to Ug99 virulent race can affect 90%-100% of wheat crop (Beard *et al.*, 2006). Ug99 is the most devastating race of *Puccinia graminis* f. sp. *tritici* and it considered as a major threat to wheat production. It first appeared in Uganda in 1999 and now has spread throughout East Africa, Yemen, Sudan, and Iran. Its spread has now been predicted toward North Africa, Middle East Asia, and beyond, raising serious concerns of major epidemics that could destroy wheat crops in various areas (Singh *et al.*, 2008). Two variant strains of Ug99, TTKST and TTSSK, were detected in Kenya in 2006 and 2007, indicating the evolution of

Ug99. In 2007, there was a severe epidemic in some regions of Kenya by TTKST, and half of the global wheat germplasm that was resistant to Ug99 appeared to be susceptible to this variant (Singh *et al.*, 2008). To date, 50 stem rust resistance genes have been reported in wheat and its wild relatives (McIntosh *et al.*, 2003). Most of these genes are specific to pathogen race except *Sr2*, which is race-non specific and provides durable resistance (McIntosh *et al.*, 1995; Singh *et al.*, 2006). *Sr2* confers slow rusting, which may not substantially reduce yield losses under severe epidemics. Therefore, deployment of *Sr2* with other rust resistance minor genes, commonly called *Sr2*-complex, can provide resistance against most of the stem rust races, including Ug99 (Singh *et al.*, 2006).

Molecular markers provide an efficient way to address problems faced in conventional breeding methods. Rust resistance genes can be tagged with tightly linked DNA markers and selection based on these markers improves the efficiency of breeding programs (Todorovska *et al.*, 2009). With the advent of marker-assisted selection (MAS), gene pyramiding, in which genes identified in different genotypes are deployed into a single cultivar that contains desired alleles at more than one locus, has become efficient (Joshi and Nayak, 2010). Several DNA markers linked to various stem rust resistance genes in wheat have been identified and developed. The genes include *Sr2* (Spielmeyer *et al.*, 2003; Hayden *et al.*, 2004), (Tsilo *et al.*, 2007), *Sr24* (Mago *et al.*, 2005; Olson *et al.*, 2010), *Sr25* (Liu *et al.*, 2010), *Sr26* (Mago *et al.*, 2005; Liu *et al.*, 2010), *Sr31* (Das *et al.*, 2006), *Sr35* (Zhang *et al.*, 2010), *Sr36* (Tsilo *et al.*, 2008), *Sr38* (Helguera *et al.*, 2003), *Sr39* (Gold *et al.*, 1999), *Sr40* (Shuangye *et al.*, 2009), *SrCad* (Hiebert *et al.*, 2011), *SrWeb* (Hiebert *et al.*, 2010), *Sr51* (Liu *et al.*, 2011), *Sr52* (Qi *et al.*, 2011), and *Sr53* (Liu *et al.*, 2011).

There is limited information on the presence/absence of major stem rust resistance genes in local Egyptian genotypes. This information will aid wheat breeders in selecting markers to use in MAS and gene pyramiding to enhance durability of stem rust resistance. This study aimed to identify new sources of resistance to stem rust in 7 Egyptian cultivars and 4 CIMMYT lines.

MATERIALS AND METHODS

Wheat genotypes used in this study included a set of 25 stem rust resistance genes (*Sr2*, *Sr6*, *Sr16*, *Sr17*, *Sr18*, *Sr21*, *Sr22*, *Sr23*, *Sr24*, *Sr25*, *Sr26*, *Sr27*, *Sr28*, *Sr29*, *Sr30*, *Sr31*, *Sr32*, *Sr33*, *Sr34*, *Sr35*, *Sr36*, *Sr37*, *Sr38*, *Sr39* and *Sr40*). Seven local Egyptian wheat cultivars (Sakha-93, Sids-12, Sids-13, Gemmiza-10, Gemmiza-11, Misr-1 and Misr-2) and four lines (Line-6043, Line-6086, Line-6107 and Line-6085) from the International Maize and Wheat Improvement Center (CIMMYT) were tested under Egyptian condition.

Stem rust evaluation under greenhouse and field conditions:

Evaluation of selected accessions for seedling host response against *Puccinia graminis* Pers. f. sp. *tritici* was conducted in the greenhouse of Cereal Dis. Div., Plant Pathol. Inst. at Giza during 2012/2013. Method for inoculum preparation, inoculation, incubation, and disease estimation were as

described by Tervet and Cassel (1951) and Stakman *et al.* (1962). Seedlings with infection type (IT) based on scale (0-4) was recorded 15 days after inoculation. Seedlings with 0, 0;, 1 and 2 scores were considered resistant and those with high IT scores 3 and 4 were classified as susceptible. Adult plant resistance was evaluated on the same set of materials at two locations (Kafr El-hamam and Sakha research stations) during 2012/2013 growing season, the recommended agricultural practices were applied. Disease severity was assessed using the modified Cobb 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 were extracted from leaves following the protocol described by Mago *et al.* (2005). 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, Korea Cat. No. 17371) according to manufacturer's instructions. The eluted DNA was stored at -20 °C.

PCR mixture:

PCR reaction was conducted in reaction volume of 25 µl. Each PCR mixture (25 µl) contains 1 µl of 25 ng nucleic acid, 1 µl of each primer (10 pmol), 12.5 µl of GoTag (R) 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. DNA bands were visualized using a UV Tran illuminator. Sequences of primers are listed in Table (1).

Table 1: List of stem rust resistance genes sequences of primers.

| Gene | marker | Primer sequence | Reference |
|------|---------|--|---------------------------|
| Sr2 | csSr2 | csSr2-F 5'- CAA GGG TTG CTA GGA TTG GAA AAC -3' csSr2-R 5'- AGA TAA CTC TTA TGA TCT TAC ATT TTT CTG -3' | Mago <i>et al.</i> , 2011 |
| Sr24 | Sr24#12 | Sr24#12-F 5'- CAC CCG TGA CAT GCT CGT A -3' Sr24#12-R 5'- AAC AGG AAA TGA GCA ACG ATG T -3' | Mago <i>et al.</i> , 2005 |
| Sr26 | Sr26#43 | Sr26#43-F 5'- AAT CGT CCA CAT TGG CTT CT -3' Sr26#43-R 5'- CGC AAC AAA ATC ATG CAC TA -3' | Mago <i>et al.</i> , 2005 |
| Sr31 | lag95 | lag95-F 5'- CTCTGTGGATAGTTACTTGATCGA 3' lag95-R 5'- CCTAGAACATGCATGGCTGTACA 3' | Mago <i>et al.</i> , 2002 |

RESULTS

The objective of this study was to identify *Sr* resistance genes (*Sr2*, *Sr24*, *Sr26* and *Sr31*) in 7 local Egyptian wheat cultivars and in 4 lines from CIMMYT, so as to facilitate future *Sr* gene pyramiding against stem rust.

Seedling and adult tested monogenic lines and local cultivars against stem rust:

Data in Table (2) indicated that, at seedling stage the monogenic lines that exhibited high ITs (3 and 4) were *Sr6*, *Sr18*, *Sr21*, *Sr28*, *Sr36* and *Sr40* with efficacy of 20%, followed by *Sr16*, *Sr17*, *Sr22*, *Sr30* and *Sr34* with efficacy of 40%. These monogenic lines were not effective against five races under greenhouse conditions. While monogenic lines which exhibited low ITs (0, 0;, 1 and 2) indicated that *Sr2*, *Sr24*, *Sr25*, *Sr26*, *Sr31*, *Sr32*, *Sr37*, *Sr38*

and Sr39 gave high efficacy (100%), which were the most effective genes against five races, followed by Sr27 and Sr29 (80%).

Table 2: Response of some monogenic lines to stem rust reaction at seedling and adult stage.

| No. | Genes | Infection type to five pathotypes at seedling stage in Giza greenhouse | | | | | Efficacy % | Disease severity at adult stage in two location** | |
|-----|-------|--|-------|-------|-------|-------|------------|---|---------------|
| | | RSPCB | THRTC | TTTPB | BBBCC | RHTTC | | Sakha | Kaferel hamam |
| 1 | Sr2 | 1 | 2 | 2 | 1 | 2 | 100 | TrMR | TrMR |
| 2 | Sr6 | 3 | 4 | 4 | 0 | 4 | 20 | 10S | 40S |
| 3 | Sr16 | 0 | 4 | 4 | 1 | 4 | 40 | 30S | 60S |
| 4 | Sr17 | 3 | 1 | 4 | 0 | 4 | 40 | 30S | 40S |
| 5 | Sr18 | 0 | 3 | 4 | 4 | 4 | 20 | 20S | 50S |
| 6 | Sr21 | 3 | 4 | 4 | 1 | 4 | 20 | 30S | 60S |
| 7 | Sr22 | 2 | 4 | 4 | 0 | 4 | 40 | 20S | 50S |
| 8 | Sr23 | 1 | 0 | 4 | 0 | 4 | 60 | 10S | 30S |
| 9 | Sr24 | 1 | 1 | 1 | 0 | 1 | 100 | 10R | 10R/MR |
| 10 | Sr25 | 0 | 1 | 0 | 0 | 2 | 100 | 5MR | 10MR |
| 11 | Sr26 | 2 | 2 | 1 | 0 | 2 | 100 | 10MR | 20MR |
| 12 | Sr27 | 2 | 4 | 0 | 0 | 1 | 80 | 30MR | 40MR |
| 13 | Sr28 | 3 | 4 | 4 | 0 | 4 | 20 | 30S | 50S |
| 14 | Sr29 | 0 | 0 | 4 | 0 | 1 | 80 | 10S | 50S |
| 15 | Sr30 | 4 | 0 | 4 | 0 | 4 | 40 | 10S | 30S |
| 16 | Sr31 | 0 | 0 | 0 | 0 | 1 | 100 | 10R | 20MR |
| 17 | Sr32 | 2 | 0 | 2 | 2 | 1 | 100 | 20MR | 40MR |
| 18 | Sr33 | 3 | 0 | 0 | 0 | 4 | 60 | 5 MS | 10MS |
| 19 | Sr34 | 2 | 4 | 4 | 0 | 4 | 40 | 20S | 40S |
| 20 | Sr35 | 2 | 0 | 4 | 0 | 3 | 60 | 10S | 20S |
| 21 | Sr36 | 3 | 4 | 4 | 0 | 4 | 20 | 5S | 20S |
| 22 | Sr37 | 1 | 1 | 1 | 0 | 2 | 100 | 10S | 30S |
| 23 | Sr38 | 2 | 0 | 2 | 2 | 0 | 100 | 5MS | 5S |
| 24 | Sr39 | 2 | 0 | 0 | 0 | 0 | 100 | 10S | 50S |
| 25 | Sr40 | 2 | 4 | 4 | 4 | 4 | 20 | 30S | 40S |

Infection type (IT) based on a 0-4 scale.

** R = resistant, MR = moderately resistant, Tr = Trace, S = susceptible and MS = moderately susceptible.

Table 3: Response of 7 Egyptian wheat cultivars and 4 lines from CIMMYT to stem rust at seedling and adult stages under Egyptian conditions.

| No. | Entries | Infection type of two races at seedling stage* | | Disease severity at adult stage in two locations** | |
|-----|-------------|--|-------|--|---------------|
| | | TTTPB | RHTTC | Sakha | Kaferel hamam |
| 1 | Sakha-93 | 4 | 3 | 10S | 20S |
| 2 | Sids-12 | 2 | 1 | 5S | 20S |
| 3 | Sids-13 | 2 | 2 | 0 | TMR |
| 4 | Gemmeiza-10 | 1 | 2 | 10S | 20S |
| 5 | Gemmeiza-11 | 2 | 2 | 0 | 0 |
| 6 | Misr-1 | 2 | 2 | 10S | 20S |
| 7 | Misr-2 | 3 | 2 | 10S | 30S |
| 8 | Line-6043 | 2 | 2 | 0 | 0 |
| 9 | Line-6086 | 1 | 2 | 0 | 0 |
| 10 | Line-6107 | 3 | 3 | 5R-MR | 10MR |
| 11 | Line-6085 | 2 | 2 | 0 | 0 |

Infection type (IT) based on a 0-4 scale.

** R = resistant, MR = moderately resistant, Tr = Trace, S = susceptible and MS = moderately susceptible

Data in Table (2) indicated also that, at adult stage the monogenic lines (Sr24, Sr25, Sr26 and Sr31) exhibited mostly R to MR infection

responses with relatively low disease severity in the field at both locations. Susceptible infection response with high disease severity was observed on the monogenic lines (*Sr6*, *Sr16*, *Sr17*, *Sr18*, *Sr21*, *Sr22*, *Sr23*, *Sr28*, *Sr29*, *Sr30*, *Sr34*, *Sr35*, *Sr36*, *Sr37*, *Sr38*, *Sr39* and *Sr40*) at both locations.

On other hand, resistances of the eleven genotypes of wheat against stem rust at seedling and adult stages at two locations were shown in Table (3). Sids-13, Gemmeiza-11, Line-6043, line-6085 and line-6086 showed resistance at both stages. Meanwhile, Sids-12, Gemmeiza-10, Misr-1 and Misr-2 were resistant at seedling but susceptible at adult stage. On the other hand the line-6107 was susceptible at seedling stage but was resistant at adult stage. The rest of the cultivars were susceptible at both stages.

Molecular markers:

Seven Egyptian wheat cultivars (Sakha-93, Sids-12, Sids-13, Gemmeiza-10, Gemmeiza-11, Misr-1, and Misr-2) and four lines (Line-6043, line-6086, line-6107 and line-6085) from the CIMMYT and four monogenic lines (*Sr2*, *Sr24*, *Sr26* and *Sr31*) were chosen as resistant plant materials (*Sr* genes) to detect stem rust resistance genes using molecular markers. In this study, *Sr* specific primer was used to detect the presence/absence of *Sr* gene. The polymorphic survey revealed that the marker for *Sr2* was identified as a fragment of 310bp in eleven genotypes as shown in Figure (1). The marker for *Sr24* was identified as a fragment of 500bp in three genotypes only (Sakha-93, Misr-1 and line-6085), while other listed genotypes did not show the presence of *Sr24* Fig. (2). The polymorphic screening of the eleven genotypes revealed that, the marker for *Sr26* was identified as a fragment of 250bp in nine cultivars *i.e.* Sakha-93, Sids-12, Sids-13, Gemmeiza-10, Gemmeiza-11, Misr-1, Misr-2 and two lines *i.e.* line-6043 and line-6107. While two tested genotypes line-6068 and line-6085 did not show the presence of *Sr26* Fig.(3). The marker for *Sr31* was identified as a fragment of 1100bp in all Egyptian cultivars except cv. Sids-13 . While all CIMMYT lines did not show the presence of *Sr31* except line 6043 (Fig. 4).

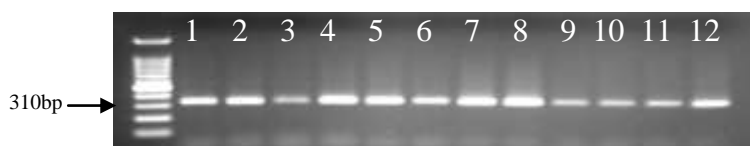


Fig.1. Marker *csSr2* tested on diverse wheat genotypes and run on an agarose gel. Lanes: (1) monogenic *Sr2*, (2) Sakha-93, (3) Sids-12, (4) Sids-13, (5) Gemmeiza-10, (6) Gemmeiza-11, (7) Misr-1, (8) Misr-2, (9) Line-6043,(10) Line-6068, (11) Line-6107 and (12) Line-6085. The arrow showed the fragment which is associated with *Sr2*.



Fig.2. Marker *Sr24#12* tested on diverse wheat genotypes and run on an agarose gel. Lanes: (1) monogenic *Sr24*, (2) Sakha-93, (3) Sids-12, (4) Sids-13, (5)

Gemmeiza-10, (6) Gemmeiza-11, (7) Misr-1, (8) Misr-2, (9) Line-6043, (10) Line-6068, (11) Line-6107 and (12) Line-6085. The arrow showed the fragment which is associated with Sr24.



Fig.3. Marker Sr26#43 tested on diverse wheat genotypes and run on an agarose gel. Lanes: (1) monogenic Sr26, (2) Sakha-93, (3) Sids-12, (4) Sids-13, (5) Gemmeiza-10, (6) Gemmeiza-11, (7) Misr-1, (8) Misr-2, (9) Line-6043, (10) Line-6068, (11) Line-6107 and (12) Line-6085. The arrow showed the fragment which is associated with Sr26.

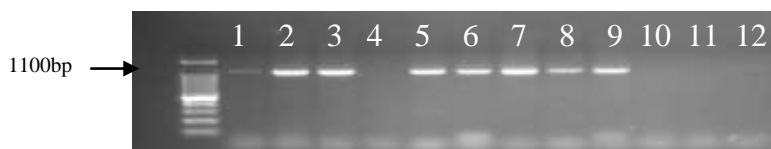


Fig.4. Marker iag95 tested on diverse wheat genotypes and run on an agarose gel. Lanes: (1) monogenic Sr31, (2) Sakha-93, (3) Sids-12, (4) Sids-13, (5) Gemmeiza-10, (6) Gemmeiza-11, (7) Misr-1, (8) Misr-2, (9) Line-6043, (10) Line-6068, (11) Line-6107 and (12) Line-6085. The arrow showed the fragment which is associated with Sr31.

Table 4. Sr genes detected with PCR based markers in 7 Egyptian wheat cultivars and 4 CIMMYT wheat lines.

| Genotypes | Sr2 | Sr24 | Sr26 | Sr31 |
|-------------|-----|------|------|------|
| Sakha-93 | + | + | + | + |
| Sids-12 | + | - | + | + |
| Sids-13 | + | - | + | - |
| Gemmeiza-10 | + | - | + | + |
| Gemmeiza-11 | + | - | + | + |
| Misr-1 | + | + | + | + |
| Misr-2 | + | - | + | + |
| Line-6043 | + | - | + | + |
| Line-6068 | + | - | - | - |
| Line-6107 | + | - | + | - |
| Line-6085 | + | + | - | - |

(+) = presence of Sr gene in wheat cultivars and (-) = absence of Sr gene in wheat genotype

DISCUSSION

The new races overcome the resistance of widely deployed stem rust resistance genes, especially *Sr31*. Development of resistant wheat varieties is the most economic method for controlling the disease. To date, more than 45 stem rust resistance *Sr*'s (genes) (McIntosh *et al.*, 2003) were identified. In Egypt during many growing seasons, samples were collected from trap nurseries and farmer fields. Gene pyramiding using conventional method is difficult and time consuming because it requires simultaneous tests of the same wheat breeding materials with several different rust races before making selection. The development of molecular markers for specific stem rust genes allows the 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). In the past 50 years, a number of *Sr* genes have been identified and incorporated into wheat genomes through chromosome engineering. *Sr22*, *Sr25*, *Sr27*, *Sr32*, *Sr33*, *Sr35*, *Sr37*, *Sr39*, *Sr40*, *Sr44*, *Sr45*, and *Sr46* and few unnamed genes are still resistant to Ug99 and its derivatives (Xu *et al.*, 2008).

The present study was conducted to determine the reaction type of the genotypes varies from resistant (R) to susceptible (S). The seedling and adult tests clustered the wheat genotypes into four main categories; 1) resistant at both seedling and adult plant stages monogenic lines (*Sr24*, *Sr25*, *Sr26*, *Sr27*, *Sr31* and *Sr32*), 2) resistant only at adult stage monogenic lines (*Sr27*), 3) high susceptibility at both seedling and adult stages monogenic lines (*Sr6*, *Sr16*, *Sr17*, *Sr18*, *Sr21*, *Sr22*, *Sr23*, *Sr28*, *Sr29*, *Sr30*, *Sr33*, *Sr34*, *Sr35*, *Sr36* and *Sr40*) and 4) resistant only at seedling stage monogenic line (*Sr37*, *Sr38* and *Sr39*). On other hand wheat cultivars and lines of Sids-13, Gemmeiza-11, Line-6043, line-6085 and line-6086 showed resistance at both stages. Meanwhile, Sids-12, Gemmeiza-10, Misr-1 and Misr-2 were resistant at seedling but susceptible at adult stage. The rest of the cultivars were susceptible at both stages. The lines which show resistance at both seedling and adult plant stages are mainly due to major genes.

Sr2 found in all tested genotypes with a diagnostic band of 310bp amplified and indicate the presence of *Sr2* gene. Although this gene alone is capable of reducing the level of infection and slow rusting resistance gene reported by (Singh *et al.*, 2011). The presence of *Sr2* in some of the land races (Haile *et al.*, 2013) also strengthen this fact and showed that Ethiopian cultivated tetraploid wheat accessions are still good sources of stem rust resistance. *Sr2*, APR to Ug99 races conferred by *Sr2* on chromosome 3BS was validated in at least six recombinant inbred line populations characterized by CIMMYT (Bhavani *et al.*, 2011), as well as in several other research efforts. An improved marker for *Sr2* is available (Mago *et al.*, 2011), and continued efforts to develop "perfect" markers are underway. Significant interaction of markers linked to *Sr2* with markers linked to other resistance loci was detected in multiple association mapping panels (Yu *et al.*, 2011 and 2012).

Many CIMMYT and other varieties internationally contain this gene in combination with other sources of resistance. *Sr2* is considered by many to be the foundation of APR breeding efforts (Liu *et al.*, 2010). DNA markers for *Sr25* were identified enabling selection of this *Thinopyrum ponticum*-derived alien resistance on chromosome 7DL. CIMMYT germplasm containing *Sr25* and presumably Lr19 in combination with *Sr2* was recently released in Afghanistan (Muqawim 2009), Egypt (Misr-1, and Misr-2) and Pakistan (NR356).

Sr24 resistance gene confers resistance to stem rust but not to its variants. Our results showed the absence of this gene in 9 genotypes and its presence in one local Egyptian cultivar (Sakha-93) and one line from CIMMYT (Line-6085). Therefore, deployment of this gene in the Egyptian cultivars should be encouraged. This will provide resistance to other

prevalent *Puccinia graminis* f. sp. *tritici* races and may provide residual resistance to its variants as suggested by Knott (2008). Moreover, *Sr24* gene is also useful due to its linkage with Lr24. Klindworth *et al.* (2012) reported the occurrence of this gene in U.S. winter wheat, which can be used as source of *Sr24*.

Sr26 gene marker which produced a 250bp band was observed in nine genotypes and was absent in two genotypes with *Sr26*. New translocation stocks with reduced *Thinopyrum ponticum* chromatin containing *Sr26* on chromosome 6AL were identified. PCR-based markers that allow for routine detection can be used to identify these smaller translocations (Liu *et al.*, 2010). Australian varieties with the original translocation have been grown for over 30 years (McIntosh *et al.* 1995). Deployment of *Sr26* is not documented in the rest of the world presumably due to real or perceived evidence of linkage drag preventing realization of other traits, but many programs are currently using the new translocation derivatives in resistance breeding efforts.

Results from *iag95* marker were used to detect *Sr31*. Marker resulted in an 1100bp fragment which was observed in 7 genotypes and was absent in the remaining four genotypes. Before the emergence of Ug99, stem rust resistance was maintained mainly by *Sr31* in most of the countries around the world except Australia (Singh *et al.*, 2008). Our results showed the presence of *Sr31* in these genotypes indicating that *Sr31* may be effective against Egypt stem rust races. Pretorius *et al.* (2012) reported that marker *iag95* has been successfully validated on South African germplasm also.

Marker assisted analysis of these resistance genes is important to Egypt breeders because they cannot directly evaluate resistance to Ug99 and associated foreign races in their breeding. Using molecular markers in pyramiding 2-3 genes of *Sr24*, *Sr26*, *Sr31* and *SrR* has been reported (Mago *et al.*, 2011). *Sr22*, *Sr26*, and *Sr35* conferred resistance to Ug99 and other important races (Singh *et al.*, 2011). Markers for these genes could then aid in selecting APR, pyramiding R-genes and in combining APR genes with R-genes (Yu *et al.*, 2011). Good example are varieties released in Egypt, Afghanistan and Pakistan whose resistance is based on single race-specific gene (*Sr25*) and slow rusting resistance gene (*Sr2*) (Singh *et al.*, 2011).

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تعريف وتقييم مصادر المقاومة لصدأ الساق في بعض التراكيب الوراثية المصرية والسيمت بالطرق التقليدية والدلائل الجزيئية عبدالعزیز عبدالناصر محمد ابوعلی ، عاطف عبد الفتاح شاهین ، دعاء النجار و جمالات هرماس

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صدأ ساق القمح المتسبب عن بكسينيا جبراميس ترتيساي هو واحد من أكثر الأمراض المدمرة للقمح. الأصناف المقاومة ابسط طريقة عملية وفعالة واقتصادية لمكافحة المسبب المرضي. تطور الدلائل الجزيئية تساعد علي معرفة الجينات المقاومة. الهدف من تلك الدراسة هو معرفة جينات المقاومة الفعالة ضد صدأ الساق في طوري البادرة والبلوغ ومعرفة جينات المقاومة في بعض التراكيب الوراثية بواسطة الدلائل الجزيئية. فقد تمت تلك الدراسة على احدى عشر من التراكيب الوراثية المختلفة بواسطة الدلائل الجزيئية. حيث أوضحت نتائج الدراسة في طور البادرة والنبات البالغ إلي استجابة جينات المقاومة لصدأ الساق والمدخلات الوراثية للإصابة إلى أربعة فئات رئيسية الأولى منها تظهر مقاومة المدخلات في كل من طوري البادرة والنبات البالغ وكانت تلك الجينات هي *Sr32* , *Sr31* , *Sr26* , *Sr25* , *Sr24* والثانية تظهر المقاومة في طور النبات البالغ فقط جين *Sr27* والفئة الثالثة وهي جينات *Sr18* , *Sr17* , *Sr16* , *Sr6* , *Sr36* , *Sr35* , *Sr34* , *Sr33* , *Sr30* , *Sr29* , *Sr28* , *Sr23* , *Sr22* , *Sr21* و *Sr40* والتي كانت قابلة للإصابة بشدة في طوري البادرة والنبات البالغ. أما عن الفئة الأخيرة أظهرت مقاومة في طور البادرة فقط وهي *Sr39* , *Sr38* , *Sr37* وعن الأصناف التجارية وباقي المدخلات الوراثية أظهرت بعضها مقاومة في كل من طور البادرة وطور النبات البالغ مثل سدس 13 ، جميزة 11 والسلالة 6043 والسلالة 6085 والسلالة 6086 بينما سدس 12 وجميزة 10 ومصر 1 ومصر 2 مقاومة في طور البادرة ولكنها كانت قابله للإصابة في طور النبات البالغ.

وفي هذه الدراسة أيضا تم فحص احدى عشر مدخل وراثي مع أربعة دلائل جزيئية للكشف عن وجود أو عدم وجود جينات المقاومة لصدأ الساق وهي *Sr2* , *Sr24* , *Sr26* و *Sr31* حيث الجين *Sr2* كان موجود في جميع المدخلات الوراثية المختبرة في حين *Sr24* موجود في صنفين من الأصناف المصرية ومدخل وراثي واحد فقط من أصناف السيمت. وعن *Sr26* تم تحديده في تسعة مدخلات وراثية ولم يشاهد

في باقي الأصناف المختبرة وعن الجين *Sr31* تم تحديده في سبعة مدخلات وراثية بينما لم يشاهد في باقي المدخلات .
وهذه النتائج تشكل أساس لدراسة المقاومة وخاصة في المصادر الغير معرف بها جينات المقاومة التي تساعد المربي في الحصول علي اصناف مقاومه بواسطة الدلائل الجزئية.