Identified SSR Marker Linked to Leaf, Stripe and Steam Rust Resistance Genes in Some High Yield Potential Bread and Durum Wheat varieties

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ABSTRACT

This study was conducted to identify SSR markers linked to leaf, stripe and stem rusts resistance genes *Lr34*, *Lr 19* and *Lr 47*, *Yr 10* and *Yr 15* and *Sr 2*, *Sr12*, *Sr 24*, *Sr 26#43* and *Sr 25* to be used as markers for identifying the rust resistance genes in seventeen Egyptian bread and durum wheat varieties and two promising lines produced from doubled haploid plants. Bread wheat varieties (Misr 1, Giza 168, Gemmieza 10, Nubaria 1, Sahel 1, Sids 13, Sids 4, Sakha 94, Gemmeiza 11, Giza 171, Gemmeiza 9, Sids 1 and two doubled haploid promising lines DH 2 and DH3) and durum wheat varieties (Bani Suef 5, Bani Suef 6 and Sohag 3). The materials were planted in the two successive growing seasons 2015/2016 and 2016/2017 at El-Giza research station to determine some characters, i. e., No. of kernels/spike, No. of spikes/m^2, 1000-kernel weight(g) and grain yield Ardab/faddan. Moreover, materials were tested to rust diseases in Sakha and Nubaria stations in 2015/2016 and 2016/2017. Significant differences were found among varieties in the two growing seasons, in all characters in this study. DH#3 promising line recorded the highest value in No. of spikes/m^2 and grain yield ardab/faddan in the two growing seasons. Misr 1, Giza 171, DH#2 and DH#3 had no infection of rust reaction in the two growing seasons for the three types of rusts, but Sids 13 and Nubaria 1 were 5MS (moderate susceptible) for Yr rust and 10 MS for *Lr* rust. However, *Sr* rust reaction zero no infection in all genotypes. *Yr 10* and *Yr 15* stripe rust resistant gene used in this study, positive molecular marker Linked to *Yr 10* detected in Misr1, Sids 1and DH3, while *Yr 15* was detected in Misr 1, Giza 168, Sahel 1, Sids 4, Sids 1, Bani Suef 5, Bani Seif 6, DH#2 and DH#3. *Lr 34* leaf rust gene was found in Sids 13, Sakha 94 and DH#3, while *Lr 47* gene was detected in Misr 1, DH#2 and DH#3, but *Lr 19* was detected in any genotype. *Sr 2* durable resistant stem rust gene was detected in all genotypes expect Sids 1, moreover *Sr 26#43* detected in all genotypes too expect Sids 4 variety. *Sr 12* gene was detected in Misr 1, Giza 171, Gemmeiza 9 and DH# 3. All genotypes had no *Sr 24*, While *Sr 25* resistance gene was detected in Misr 1, Giza 171 and DH#3 genotypes. Dedication of more than one gene in any variety is useful for durable resistance. Seven rust genes were detected in Misr 1 for the three rusts and eight genes were found in promising line DH#3, which caused resistant to rust. So, Misr 1 and DH#3 are considered as promising bread wheat high yielding genotypes and resistant to rust diseases can which be used in breeding program, as pre breeding in hybridization program.

Keywords: bread, durum, Wheat, resistance gene, SSR marker, yield, rust diseases, rust reaction.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important crops in the world. It is the staple food of 43 countries, and about one-third of the world's population depends on wheat for food (Encyclopedia Britannica online). Increasing grain yield of wheat crop is considered one of the most important national goals in Egypt to face the decreased self-sufficient which result from rapid increasing of population. Only, 65% of self-sufficient from wheat were produced in 2016/2017 season. The total production was estimated by 8.757 million tons resulted from 2.92 million Faddan (**The Agricultural Economic and Statistics Department, Ministry of Agriculture, 2017**). Hard efforts have been done to increasing the total wheat production. This increasing could be achieved by vertical or horizontal expansion. Leaf rust (brown rust), stripe rust (yellow rust), and stem rust (black rust) for wheat are caused by *Puccinia triticina Eriks*., *Puccinia striformis*, and *Puccinia graminis f. sp. tritici* respectively. Rusts are the most important plant diseases that threaten wheat vertical expansion in Egypt under severe infection, rust pathogen can destroy the entire susceptible wheat crop and make big economic losses (**Marsalis and Goldberg, 2006**). The most common rust is leaf rust, it occurs on the leaf blades, and leaf sheaths can also be infected under favorable conditions, with high inoculation densities, and extremely susceptible cultivars. Losses of grain yield can be increased to 30% or more. Stem rust is also known as black rust due to the abundant production of shiny black teliospores. Stem rust is favored by humid warmer condition. It may decrease grain yield by 50%, these losses can be achieved to 100% with susceptible cultivars. Stripe or yellow rust is principally occurred under low temperature conditions with temperature degree ranged from 2 to 15˚C. Losses under extreme situations can be achieved to 100% losses (**Roelfs et al 1992**). Plant breeders work to develop cultivars with genetic resistance against rust disease. Breeding for disease resistance is the most effective method to crop protection. Selection of cultivars with single-gene resistance has short time resistance as results to the ability of pathogen to produce single step mutations. In addition, it is difficult to detect a single gene of interest within a complex background of other

resistance genes. Hence, using combinations of resistance genes is the best method for achieving to adequate genetic control of rust diseases for wheat (**Roelfs, 1989**). Consequently, plant pathologists and plant breeders have a priority to develop resistance varieties with high-yielding capacity through pyramiding effective resistance genes with durable resistance. This gene pyramiding strategy, also known as multigenic resistance. It has been used to increase the durability of resistance. Using combinations of genes has been suggested as the best method for genetic control of rust (**Roelfs, 1988** and **Savitha et al., 2016**). Using selection application of molecular techniques and Marker-Assisted (MAS) can help in breeding program to reduce the period of time and help in pyramiding resistance genes into single cultivar for durable resistance in segregating populations, **Qi et al.** (**2015**) and **Chhuneja et al. (2011)**. Resistance to rust can be divided into two categories, qualitative and quantitative resistance, **Qi et al.** (**2015**).

The objective of this study was to evaluate the selected genotypes for their resistance to rust diseases and identify genes linked to this resistance by using SSR marker.

Materials and Methods

A field experiment was carried out at the Agricultural Research Center at El-Giza, Egypt, during the two successive seasons 2015/2016 and 2016/2017. fourteen bread (*Triticum aestivum* L.) and three durum wheat (*Triticum durum var. durum*) genotypes were used in the present study. These genotypes were selected on the basis of their genetic diversity. The pedigree of the selected genotypes is presented in Table (1). Each season, three experiments were conducted at El-Giza, Sakha, and Nubaria Agricultural Stations, Agricultural Research Center, Egypt. Seventeen cultivars were arranged in a randomized complete block design (RCBD). The plot area was 6.3 m^2 (6 rows, 3.5 m long and 30 cm apart). All recommended cultural practices were adopted. Sakha and EL-Nubaria are hot spot regions; they are subjected to severe attack of yellow, leaf, and stem rusts.

Table 1. The Name, Pedigree and Origin of the Seventeen Bread and Durum Wheat Genotypes.

Ser	Varieties	Pedigree
1	Misr1	**OASIS/SKAUZ//4*BCN/3/2*PASTOR** CMSS00Y01881T-050M-030Y-030M-030WGY-33M-0Y-0S
2	Giza168	**MRL/BUC//SERI** CM93046-8M-0Y-0M-2Y-0B-0GZ
3	Gem 10	**MAYA74/0N//160-147/3/BB/GLL/4/CHAT/5/CROW** GM5820-3GM-1GM-2GM-0GM
4	Nubaria 1	**OASIS/5*BOR95/4/CNDO/R143//ENTE/MEX175/3/CNDO/R143**
5	Sahel 1	**N.S.732/PIMA//VEE** SD735-4SD-1SD-1SD-0SD
6	Sids 13	**KAUZ//TSI/TSI/SNB** ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP-SD
7	Sids 4	**MAYA/MON//CMH74A.592/3/GIZA157*2** SD10001-2SD-3SD-2SD-0SD
8	Sakha 94	**OPATA/RAYON//KAUZ** CMBW90Y3180-0TOPM-3Y-010M-010M-010Y-10M-015Y-0Y-0AP-0S
9	Gemmeiza 11	**B0W/KVZ//7C/SERI82/3/GIZA168/SAKHA61** GM7892-2GM-1GM-2GM-1GM-0GM
10	Giza 171	**Sakha93/Gemmeiza9** Gz2003-101-1Gz-4Gz-1Gz-2Gz-0Gz
11	Gemmeiza 9	**ALD/HUAC//CMH74A.630/SX** GM4583-5GM-1GM-0GM
12	Sids 1	**HD2172/PAVON//1158.57/MAYA74** SD46-4SD-2SD-1SD-0SD
13	Beni Suef 5	**DIPPERZ/BUSHEN3** CDSS92B128-1M-0Y-0M-0Y-3B-0Y-0SD
14	Beni Suef 6	**BOOMER-21/BUSCA-3** CDSS95Y001185-8Y-0M-0Y-0B-1Y-0B-0SD
15	Sohag 3	**MEXI/MGHA/51792//DURUM6** CD21831-25H-1SH-0SH
16	DH# 2	**SAKHA94/MISR1** GZ2008-06DH2
17	DH# 3	**SAKHA94/MISR1** GZ2008-06DH3

Giza experiments were conducted to evaluate yield and yield components. Thus, number of fertile spikes/m2 was counted from each plot just before harvesting. In addition, five randomly selected main spikes were picked, threshed, and their kernels were counted. Their average was recorded to indicate the No. of kernels/spike. Moreover, ten plants were randomly selected from each plot. At harvest, six rows were harvested, weighed and threshed; and their grain yields were weighed and adjusted to arddab/faddan to indicate the grain yield. Likewise, a random sample of 1000 kernels, taken from each plot was hand counted and weighed to indicate the kernel weight. Statistical analysis of variance was made to determine the effect of seasons, genotypes and their interaction using Statistical Package for the Social Sciences (SPSS).

DNA Extraction and PCR Protocol

Total cellular DNA of parents was isolated by using Cetyl Trimethyle Ammonium Bromide (CTAB) method, **Dellaporta et al. (1983)**. PCR reactions were carried out from the purified genomic DNA with the total reaction mixture of 25 µL, 1 µL of 10 mM $MgCl_2$, 1 µL of 2.5 mM dNTPs, 0.5 µL each of forward and reverse primers (5 pmoles), 0.25 µL of 3U Taq DNA polymerase and 12.75 µL from sterile distilled water. Total reaction mixture was subjected to PCR (Biorad PTC-200) amplification with the cycling parameters according to each gene presented in Table (2).

PCR products were visualized on 4% superfine agarose gel stained with Ethidium Bromide and run in 1X TBE buffer. Low range ruler with a ladder range of 100-1,000 bp was used as a standard molecular marker with known weights as control

Table 2. Markers Name, Sequence Primers and Amplified Cycles Parameters of SSR Markers Linked To Rust Resistance Genes.

Genes	Marker Sequence	PCR program
Lr34	F: 5'GTTGGTTAAGACTGGTGATGG R: 5'TGCTTGCTATTGCTGAATAG	94ºC for 4 min, 35 cycles (94ºC for 1min, 55ºC for 1min and 72ºC for 1 min) and 72ºC for 6 min
Lr 47	F: 5'GCTGATGACCCTGACGG R: 5'TCTTCATGCCCGGTCGGGT	94ºC for 4 min, 35 cycles (94ºC for 1min, 55ºC for 1min and 72ºC for 2 min) and 72ºC for 10 min
Yr 10	F: 5'TCAAAGACATCAAGAGCCGC R: 5'TGGCCTACATGAACTCTGGAT	94ºC for 3 min, 30 cycles (94ºC for 1min, 64ºC for 45 sec and 72ºC for 1 min) and 72ºC for 5 min
Yr 15	F: 5'ATTGGACGGACAGATGCT TT R: 5' AGCAGTGAGGAAGGGGATC	94ºC for 3 min, 45 cycles (94ºC for 1min, 58ºC for 1min 20 sec and 72ºC for 2 min) and 72ºC for 10 min
Sr2	R: 5'AAG GCGAAT CAA ACG GAA TA F: 5' GTT GCT TTAGGG GAA AAG CC	95ºC for 5 min, 35 cycles (94ºC for 1min, 60ºC for 45 sec and 72ºC for 2min), 72ºC for 7min and 4ºC for forever
Sr24#12	F5'- CAC CCG TGA CAT GCT CGT A R5'-AACAGGAAATGAGCA ACG ATG T	94°C, 5 min, 7 cycles, 1ºC each cycle:92°C 30 sec, 62°C 30 sec, 72°C 30 sec, 30 cycles 92°C 30 sec, 59°C 30 sec, 72°C 30 sec and 72°C 10 min
Sr26	F5'- AAT CGT CCA CAT TGG CTT CT R5'- CGC AAC AAA ATC ATG CAC TA	94°C, 3 min, 35 cycles (94°C 60 sec, 60°C 60 sec, 72°C 120 sec), and 72°C 10 min
Sr 25# Lr19	F 5'- CAT CCT TGG GGA CCT C R 5'- CCA GCT CGC ATA CAT CCA	94°C 4 min, (35 cycles 94°C 45 sec, 50°C 30 sec and 72°C 45 sec) and 72°C 7 min

Results and Discussion

The combined analysis of variance for data obtained from El-Giza location during 2015/2016 and 2016/2017 revealed insignificant effect of seasons, and the interaction between season and all studied characters. Therefore, data in table (3) represent the results of combined analysis for yield and yield components at El-Giza station.

Data recorded significant differences among wheat cultivars for grain yield. Results show that line DH#3 had the highest value of grain yield without significant different from DH#2, and Giza 171. Data in (Table 3) recorded the superiority of Sids 4 in kernels weight. Meanwhile, promising line DH#3 had the highest number of spikes per square meter without significant difference from DH#2 Recorded data showed that highest No. of kernels/spike from Sids 4 without significant difference from DH#2 and DH#3, Giza 171, Gemmiza 11, Misr 1, and Sids 1. Performance of wheat genotypes remains a key criterion for screening breeding materials based on their yield parameter, and yield components **Peltonen-Sainio (2007)** and **Mahmoud and Ahmed (2005).**

Table 3. Mean of grain yield (ard./fad.), 1000-kernel weight (g), No. of spikes/m^2, and No. of kernels/spike in two growing seasons 2015/2016 and 2016/2017.

Varieties	Grain yield Ard./fad.	1000kernel weight/g	No. of spike/m^2	No. of kernels /spike
Misr1	26.32	48.98	443.33	70.50
Giza168	26.32	45.00	447.50	69.67
Gem 10	24.91	47.67	373.00	59.00
Nubaria 1	24.59	44.88	353.50	54.50
Sahel 1	25.04	42.17	374.17	58.17
Sids 13	24.30	40.48	343.67	56.83
Sids 4	18.89	61.58	157.00	76.17
Sakha 94	26.76	41.33	434.34	63.67
Gemmeiza 11	27.59	51.52	430.33	72.33
Giza 171	27.92	46.53	469.50	73.00
Gemmeiza 9	26.39	47.70	431.50	62.17
Sids 1	26.72	51.93	469.50	70.00
Beni Suef 5	25.75	56.00	426.83	67.00
Beni Suef 6	25.78	51.28	395.67	65.67
Sohag 3	24.95	51.60	333.83	58.50
DH#2	28.08	50.97	494.67	73.83
DH#3	28.37	51.67	503.50	73.67
Mean	25.81	48.90	443.38	66.16
LSD 5%	0.7	3.35	18.62	6.31

Final rust severity: To evaluate seventeen released wheat cultivars for their slow rusting resistance to leaf, stem, and yellow rusts two field experiments were conducted at Sakha and Nubaria stations during 2015/2016 and 2016/2017 growing seasons. The highest types of genotypes infection for yellow rust at Nubaria and Sakha during the two growing seasons are presented in table (4). Moderate resistance was detected for Giza 168, Gemmiza 10, Sahel 1, Sids 4, and Sohag 3. While, no yellow rust infection was detected on Misr 1, Sakha 94, Gemmeiza 11, Giza 171, Gemmeiza 9, Bani Suef 5, Bani Suef 6, DH# 2 and DH# 3.

Table 4. The Highest Types of Genotypes Infections for Yellow Rust at Nubaria and Sakha During The Two Growing Seasons .

Ser	Varieties	Nubaria	Sakha	Yr
1	**Misr1**	0	0	0
2	**Giza168**	R	TrMr	Tr Mr
3	**Gem 10**	0	Mr	5Mr
4	**Nubaria 1**	TrMs	5Ms	5Ms
5	**Sahel 1**	R	TrMr	Tr MR
6	**Sids 13**	TrMs	5Ms	5Ms
7	**Sids 4**	5Mr	10Mr	10Mr
8	**Sakha 94**	0	0	0
9	**Gemmeiza 11**	0	0	0
10	**Giza 171**	0	0	0
11	**Gemmeiza 9**	0	0	0
12	**Sids 1**	10Mr	5Ms	5Ms
13	**Beni Suef 5**	0	0	0
14	**Beni Suef 6**	0	0	0
15	**Sohag 3**	TrMr	5Mr	5Mr
16	**DH# 2**	0	0	0
17	**DH# 3**	0	0	0

These fourteen cultivars could be considered as resistant cultivars. On the other hand, Nubaria 1 Sids 13, and Sids 1 were detected as susceptible cultivars.

Data in table (5) present the highest type of leaf rust infection at Nubaria and Sakha during the two growing seasons show that Misr 1, Giza 171, DH# 2

and DH# 3 were resistant cultivars. Moreover, Giza 168, Gemmiza 10, Sahel 1, Sids 4, Sakha 94, Gemmiza 11, Gemmiza 9, Beni Suef 5, Beni Suef 6, and Sohag 3 had moderate resistance. On the other hand, Nubaria 1, Sids 13, and Sids 1 were detected as susceptible cultivars.

Table 5. Highest Types of Genotypes Infections for Leaf Rust at Nubaria And Sakha During The Two Growing Seasons .

Ser	Varieties	Nubaria	Sakha	Lr
1	**Misr1**	0	0	0
2	**Giza168**	5Mr	R	5Mr
3	**Gem 10**	10Mr	5Mr	10Mr
4	**Nubaria 1**	10Ms	5Ms	10 Ms
5	**Sahel 1**	10Mr	TrMr	10Mr
6	**Sids 13**	10Ms	5Ms	10Ms
7	**Sids 4**	5Mr	R	5Mr
8	**Sakha 94**	5Mr	0	5MR
9	**Gemmeiza 11**	10Mr	0	10Mr
10	**Giza 171**	0	0	0
11	**Gemmeiza 9**	TrMr	0	TrMr
12	**Sids 1**	5Ms	5Ms	5Ms
13	**Beni Suef 5**	5Mr	R	5Mr
14	**Beni Suef 6**	10Mr	R	10Mr
15	**Sohag 3**	10Mr	5Mr	10Mr
16	**DH# 2**	0	0	0
17	**DH# 3**	0	0	0

Obtained show results in table (6) that all studied cultivars had zero type reaction for stem rust disease.

Eight linked specific primers to rust disease were used in this study to identified resistance genes in the seventeen genotypes under study.

Fig (1) showed the present resistance gene (Lr 34) in all studied cultivars. Adult plant resistance gene Lr34 may not provide adequate resistance under high disease severity. When it is present alone, it could be contributed as resistance. Leaf tip necrosis (Ltn), a morphological character, showed linkage between Lr34 and Yr18.Thus, it was suggested to be utilized, in some environments, as a morphological marker for wheat lines which carry these genes (**Singh et al., 2003**).

Table 6. Highest Types of Genotypes Infections for Stem Rust at Nubaria And Sakha During The Two Growing Seasons

Ser	Varieties	Nubaria	Sakha	Sr
1	**Misr1**	0	0	0
2	**Giza168**	0	0	0
3	**Gem 10**	0	0	0
4	**Mnubaria 1**	5Mr	0	5Mr
5	**Sahel 1**	0	0	0
6	**Sids 13**	0	0	0
7	**Sids 4**	0	0	0
8	**Sakha 94**	0	0	0
9	**Gemmeiza 11**	0	0	0
10	**Giza 171**	0	0	0
11	**Gemmeiza 9**	0	0	0
12	**Sids 1**	0	0	0
13	**Beni Suef 5**	0	0	0
14	**Beni Suef 6**	0	0	0
15	**Sohag 3**	0	0	0
16	**DH# 2**	0	0	0
17	**DH# 3**	0	0	0

Lr 34

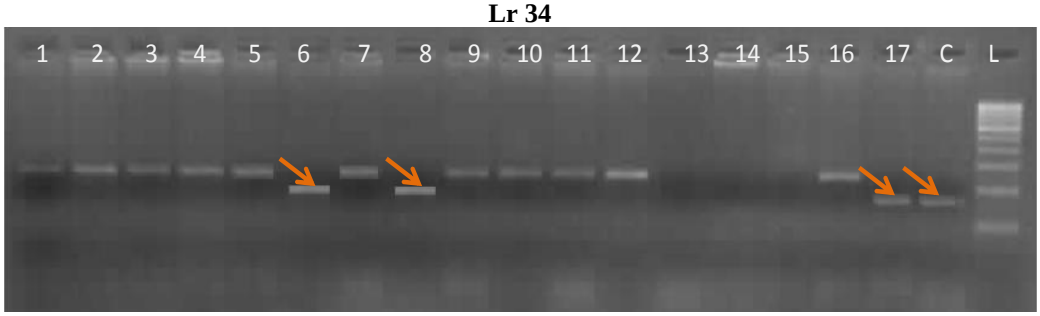

Figure (1): Lr 34 gene in the seventeen cultivars Misr 1; 2. Giza 168; 3. Gemmeiza 10; 4. Nobaria 1; 5. Sahel 1; 6. Sids 13; 7. Sids 4; 8. Sakha 94; 9. Gemmeiza 11; 10. Giza 171; 11. Gemmeiza 9; 12. Sids 1; 13. Bani Sewif 5; 14. Bani Sewif 6; 15. Sohag 3; 16. DH 4; 17. DH 5 ; +C. Positive Control; -C. Negative Control; L.Ladder

fig (2) presented that, the leaf rust resistance gene Lr47 in the seventeen varieties, which confers resistance to a wide spectrum of leaf rust strains. This gene was transferred from chromosome 7S of *Triticum speltoides* to chromosome 7A of *Triticum aestivum* (**Helguera et al. 2000**).

Lr 47

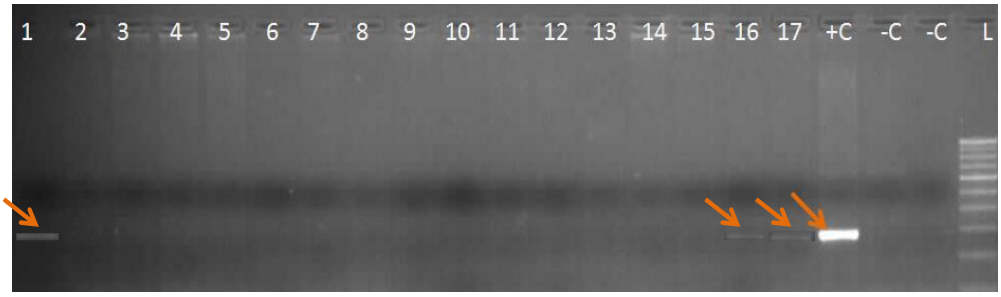

Figure (2): Lr 47 gene in seventeen cultivars 1. Misr 1; 2. Giza 168; 3. Gemmeiza 10; 4. Nobaria 1; 5. Sahel 1; 6. Sids 13; 7. Sids 4; 8. Sakha 94; 9. Gemmeiza 11; 10. Giza 171; 11. Gemmeiza 9; 12. Sids 1; 13. Bani Sewif 5; 14. Bani Sewif 6; 15. Sohag 3; 16. DH 4; 17. DH 5 ; +C. Positive Control; -C. Negative Control; L.Ladder

Yr10 is one of the catalogued seedling resistance genes fig (3), has been assigned to chromosome 1B on the basis of its linkage with Rg1 gene controlling brown glum color (**Metzger and Silbaugh, 1970**) and a second gene, *YrMoro*, has tentatively been assigned to Chromosome 4B (**Chen et al., 1995**).

Yr10

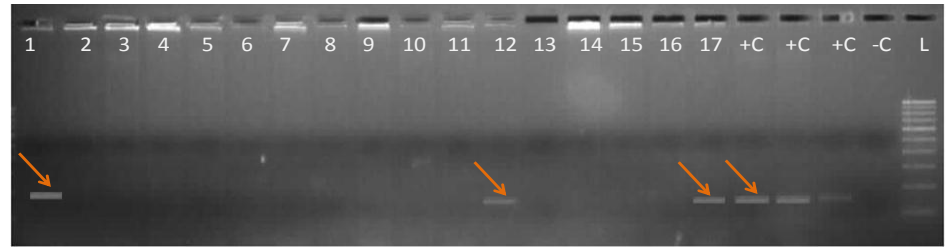

Figure (3): Yr 10 gene in seventeen varieties 1 . Misr 1; 2. Giza 168; 3. Gemmeiza 10; 4. Nobaria 1; 5. Sahel 1; 6. Sids 13; 7. Sids 4; 8. Sakha 94; 9. Gemmeiza 11; 10. Giza 171; 11. Gemmeiza 9; 12. Sids 1; 13. Bani Sewif 5; 14. Bani Sewif 6; 15. Sohag 3; 16. DH 4; 17. DH 5 ; +C. Positive Control; -C. Negative Control; L.Ladder

Yr 15 gene was showed in adult plant resistance to the stripe rust races figure (4).

Yr15

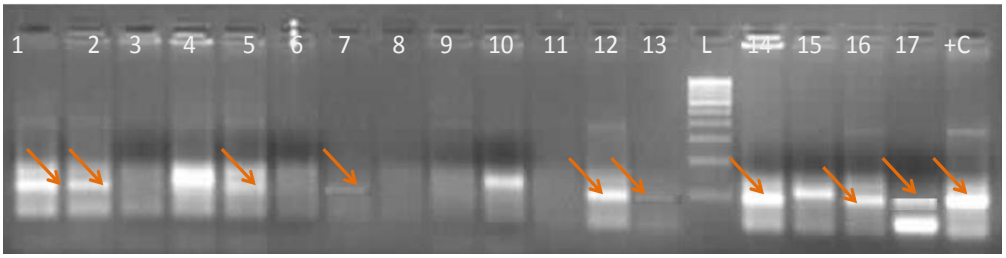

Figure (4): Yr 15 gene in seventeen cultivars 1 . Misr 1; 2. Giza 168; 3. Gemmeiza 10; 4. Nobaria 1; 5. Sahel 1; 6. Sids 13; 7. Sids 4; 8. Sakha 94; 9. Gemmeiza 11; 10. Giza 171; 11. Gemmeiza 9; 12. Sids 1; 13. Bani Sewif 5; 14. Bani Sewif 6; 15. Sohag 3; 16. DH 4; 17. DH 5 ; +C. Positive Control; -C. Negative Control; L.Ladder

Sr2 is located on the short arm of chromosome 3B and confers partial resistance only in the homozygous state (recessive resistance gene) fig (5). It was originally transferred from Yaroslavl emmer wheat into hexaploid wheat, **Singh** (2010)**.**

durable resistance Sr2

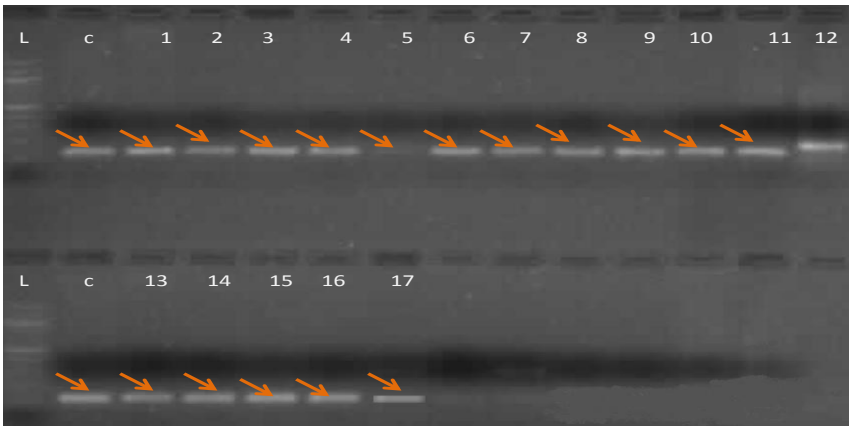

Figure (5): SR 2 gene in seventeen cultivars 1. Misr 1; 2. Giza 168; 3. Gemmeiza 10; 4. Nobaria 1; 5. Sahel 1; 6. Sids 13; 7. Sids 4; 8. Sakha 94; 9. Gemmeiza 11; 10. Giza 171; 11. Gemmeiza 9; 12. Sids 1; 13. Bani Sewif 5; 14. Bani Sewif 6; 15. Sohag 3; 16. DH 4; 17. DH 5 ; +C. Positive Control; -C. Negative Control; L.Ladder

Fig. (6) showed that, *Sr24* which offers resistance to most races of stem rust, also, STS Sr24#12 is a dominant marker that amplifies a single 500-bp band linked to *Sr24* in reference line Agent (**Rouse et al., 2014**).

STS Sr12#24

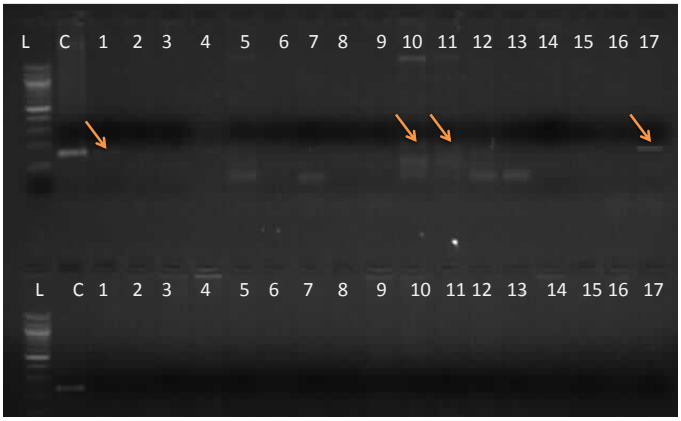

Figure (6): Sr12#24 genes in seventeen cultivars . Misr 1; 2. Giza 168; 3. Gemmeiza 10; 4. Nobaria 1; 5. Sahel 1; 6. Sids 13; 7. Sids 4; 8. Sakha 94; 9. Gemmeiza 11; 10. Giza 171; 11. Gemmeiza 9; 12. Sids 1; 13. Bani Sewif 5; 14. Bani Sewif 6; 15. Sohag 3; 16. DH 4; 17. DH 5 ; +C. Positive Control; -C. Negative Control; L.Ladder

Fig. (7) showed that Sr26#43, which originally introgressed into the distal region of the long arm of hexaploid wheat chromosome 6A , *Sr26* is one the few known major resistance genes effective against the *Sr31*-virulent race Ug99 (TTKSK) and its *Sr24*-virulent derivative (TTKST). A combination (i.e. multiplexing) of two dominant PCR markers in repulsion phase provides a diagnostic co-dominant marker for *Sr26:* Sr26#43, a dominant marker for the presence of *Sr26* and amplifies band fragment of (**207 bp) Liu et al. (2010)**.

Sr26#43

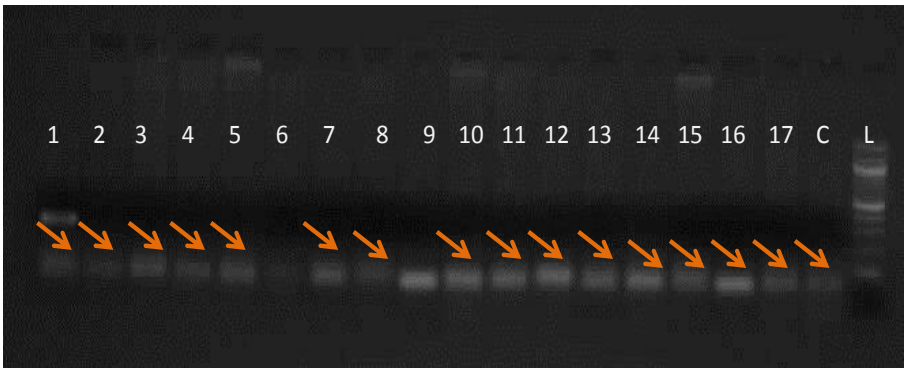

Figure (7): Sr 26#43 genes in seventeen cultivars 1 . Misr 1; 2. Giza 168; 3. Gemmeiza 10; 4. Nobaria 1; 5. Sahel 1; 6. Sids 13; 7. Sids 4; 8. Sakha 94; 9. Gemmeiza 11; 10. Giza 171; 11. Gemmeiza 9; 12. Sids 1; 13. Bani Suef 5; 14. Bani Sueif 6; 15. Sohag 3; 16. DH 4; 17. DH 5 ; +C. Positive Control; -C. Negative Control; L.Ladder

Sr25 was detected in (Fig 8), which was transferred into wheat from *Thinopyrum ponticum* (Barkworth and Dewey) and it is effective to *Ug99*. *Sr25* and the linked leaf rust resistance gene *Lr19* were translocated onto the long arm of wheat chromosomes 7D (1) and 7A (2). The dominant marker, *Gb*, amplifies band of 130 bp for this gene **lui et al. (2010)**.

Sr25 and Lr19

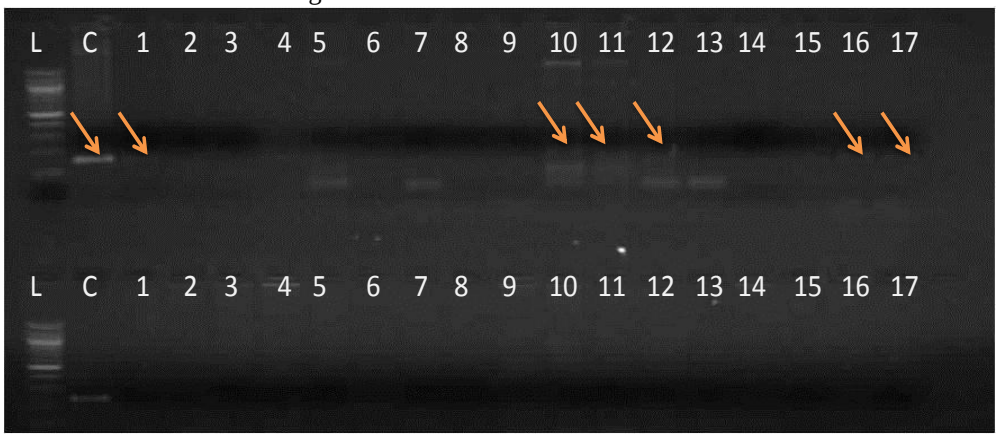

Figure (8): Sr 25#Lr 19 genes in seventeen cultivars 1 . Misr 1; 2. Giza 168; 3. Gemmeiza 10; 4. Nobaria 1; 5. Sahel 1; 6. Sids 13; 7. Sids 4; 8. Sakha 94; 9. Gemmeiza 11; 10. Giza 171; 11. Gemmeiza 9; 12. Sids 1; 13. Bani Suef 5; 14. Bani Suef 6; 15. Sohag 3; 16. DH 4; 17. DH 5 ; +C. Positive Control; -C. Negative Control; L.Ladder

Table (5) presents the results of eight SSR markers linked to rust resistance as 1=gene present and 0= gene absent. *Yr 10* positive molecular marker Linked to *Yr 10* gene detected in Misr1, Sids 1 and DH#3. *Yr 15* gene was found in Misr 1, Giza 168, Sahel 1, Sids 4, Sids 1, Bani Suef 5, Bani Seif 6, DH#2 and DH#3. *Lr 34* leaf rust gene was found in Sids 13, Sakha 94 and DH# 3, while *Lr 47* gene was detected in Misr 1, DH# 2 and DH#3, but *Lr 19* was not detected in any genotypes. *Sr 2* durable resistant stem rust gene detected in all genotypes expect Sids 1, moreover *Sr 26#43* detected in all cultivars expect Sids 4 cultivar. *Sr 12* gene was detected in Misr 1, Giza 171, Gemmeiza 9 and DH# 3. All cultivars free from *Sr 24*, while *Sr 25* resistance stem rust gene was detected in Misr 1, Giza 171 and DH# 3 resistance cultivars.

Table 5. Resistant (one) and susceptible (zero) SSR primers fragments for leaf, yellow and steam rust genes in seventeen bread and durum wheat**.**

	Genes								
Cultivars	***Lr 19***	***Lr 34***	***Lr 47***	***Yr 10***	***Yr 15***	***Sr 2***	***Sr 12***	***Sr 24***	***Sr25# Lr19***	***Sr26# 43***
Misr1	0	0	1	1	1	1	1	0	1	1
Giza168	0	0	0	0	1	1	0	0	0	1
Gem 10	0	0	0	0	0	1	0	0	0	1
Nubaria 1	0	0	0	0	0	1	0	0	0	1
Sahel 1	0	0	0	0	1	1	0	0	0	1
Sids 13	0	1	0	0	0	1	0	0	0	0
Sids 4	0	0	0	0	1	1	0	0	0	1
Sakha 94	0	1	0	0	0	1	0	0	0	1
Gemmeiza 11	0	0	0	0	0	1	0	0	0	1
Giza 171	0	0	0	0	0	1	1	0	1	1
Gemmeiza 9	0	0	0	0	0	1	1	0	0	1
Sids 1	0	0	0	1	1	0	0	0	0	1
Beni Suef 5	0	0	0	0	1	1	0	0	0	1
Beni Suef 6	0	0	0	0	1	1	0	0	0	1
Sohag 3	0	0	0	0	0	1	0	0	0	1
DH#2	0	0	1	0	1	1	0	0	0	1
DH#3	0	1	1	1	1	1	1	0	1	1

(1) = resistant gene and (0)= No Gene in genotype

In order to detect more than one gene in any variety for rust resistance, we need to identify combinations of resistance genes for durable resistance to make slow rusting **(Savitha et al. (2016)**. Seven rust genes were detected in Misr 1 resistant varietys and eight resistance genes were found in promising line DH# 3, which caused resistant to the three types of rust. So, Misr 1 and DH# 3 promising bread wheat high yielding abilils and resistant to rust diseases can be used in pyramiding resistance gene in breeding program, as pre breeding in hybridization program**.**

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التعرف على جينات المقاومة الخاصة بصدأ الأوراق والمخطط والساق فى بعض أصناف قمح الخبز والمكرونة عالية المحصول

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التعرف على جينات المقاومة للأصداء الثلاثة جينات خاصة بصدأ الأوراق وهى:*Lr19* *Lr34* و*Lr47* والبادئات الخاصة بالصدأ المخطط او الأوراق *Yr 10* و *Yr 15* والجينات الخاصة بالصدأ الأسود أو الساق *Sr 2* و*Sr 12* و*Sr 24* و*Sr 25* و *Sr 26#43* ل ١٧ صنف قمح منهم ١٤ صنف وسلالة قمح خبز (مصر ١– جيزة ١٦٨ – جميزة ١٠– نوبارية ١ – ساحل ١ – سدس ١٣ – سدس ٤ – سخا ٩٤– جميزة ١١– جيزة ١٧١ – حميزة ٩ –سدس ١ –وسلالتين مبشرتين منتجتين من نباتات الاحادية المتضاعفة DH2 – DH3) وثلاث أصناف من قمح المكرونة (بنى سويف ٥ – وبنى سويف ٦ – سوهاج ٣) بواسطة المعلمات الجزيئية باستخدام بادئات متخصصة بتكنيك SSR. تم زراعة ١٧ تركيب وراثى فى تجربة محصولية بمحطة بحوث الجيزة ٢٠١٥/٢٠١٦ و ٢٠١٦/٢٠١٧ وتم تقدير المحصول ومكوناته عدد حبوب السنبلة و عدد السنابل/م٢ و وزن ال ١٠٠ حبة بالجرام و محصول الحبوب اردب/فدان. كما تم تقييم هذه السلالات للأمراض الثلاثة بمحطتى سخا والنوبارية المنوطين باختبار جميع الأصناف والسلالات للأصداء لأمهم مصايد للأصداء فى نفس العامين, ومن أهم النتائج المتحصل عليها: تفوق السلالتين DH#2 و DH#3 معنويا فى عدد السنابل/م٢ ومحصول الحبوب أردب/فدان فى الموسمين على التوالى. وبقراءة الأصداء وجد ان الأصناف المقاومة مصرا وجيزة ١٧١ والسلالتين DH2 و DH3 لم يصابوا بالأصدأ خلال الموسمين ولكن اصيب الصنفين سدس ١٣ ونويارية ١ بالصدأ الأصفر 5MS و 10MS لصدأ الأوراق. ولم يتم الاصابة بالصدا الأسود فى جميع الأصناف محل الدراسة. بالكشف عن جينات المقاومة للصدأ الأصفر بالبادئات المتخصصة الخاصة بجين *Yr10* الذى وجد فى أصناف مصر ١ و سدس١ و السلالة DH#3 كما وجد جين Yr 15 فى مصر ١ وجيزة ١٦٨ و ساحل ١ وسدس ٤ و سدس ١ وبنى سويف ٥ وبنى سويف ٦ والسلالتين DH#2 و DH#3. وبالكشف عن جينات الخاصة بالصدأ البرتقالى, كانت جميع التراكيب الوراثية المستخدمة خالية من جين السائد *Lr19*، بينما وجد *Lr34* جين فى سدس ١٣ وسخا ٩٤ والسلالة DH3, وكذلك وجد جين *Lr47* فى مصر ١ والسلالتين DH#2 و DH#3. وبالكشف على جينات المقاومة للصدأ الأسود وجد أن جين المقاوم *Sr2* المسبب التأخير بالإصابة فى الأصناف المصرية موجود فى جميع التراكيب الوراثية المستخدمة معدا سدس١ وكذلك جينى المقاومة *Sr26#43* وجدوا ايضا فى جميع التراكيب الوراثية المستخدمة الا سدس٤. وبالكشف عن جين *Sr12* وجد فى أصناف مصر ١ وجيزة ١٧١ وجميزة ٩ والسلالة DH#3. بينما لم يكتشف وجود جين *Sr24* فى جميع التراكيب الوراثية المستخدمة فى الدراسة, ولكن اكتشف وجود جين المقاومة *Sr25* فى مصر ١ وجيزة ١٧١ والسلالة DH#3. وبوجود اكثر من جين من جينات المقاومة داخل التركيب الوراثى يعملوا معا كجين مقاوم أو تأخير الاصابة بالصدأ. لذا يجب ادخال هذه التراكيب مثل السلالة DH#3 التى تحتوى على ٨ جينات والاستفادة منها فى التهجينات فى برنامج التربية مع الأصناف التى لا تحتوى على تلك الجينات والعمل على التربية الهرمية لجينات المقاومة للأصداء.